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Two types of genetic control over the development of shape

EDMUND W. SINNOTT AND SAMUEL KAISER

(WITH TWO TEXT FIGURES)

It is obvious that the specific shapes so characteristic of organic bodies and their various parts are as readily transmitted from parent to offspring by the processes of inheritance as are any other traits, but little is definitely known as to the mechanism by which this is accomplished. The problem is of particular biological interest since the persistence of these specific shapes is one of the most distinctive attributes of living organisms. Evidence is accumulating from various sources that shape inheritance is controlled by genes similar in character to those which have been postulated for other traits. One of the present authors (1922) has shown this to be true in the case of fruit shape in *Cucurbita*, and the other, on the basis of an extensive analysis, has found a similar situation in *Capsicum* fruits. Lindstrom (1927) has determined the position of a gene for fruit shape on one of the chromosomes of *Lycopersicum*. Other instances could be cited from studies of the inheritance of shape in leaves, flowers, fruits, roots, and other plant organs.

Although shape characters thus seem to present nothing unique in their inheritance, they are necessarily related more intimately to the processes of growth and development than are many other traits. The production of any specific shape must require a highly coordinated control over growth, but the means by which such control is exercised are quite beyond our present knowledge. Shape traits are probably no more complex in character than others, but they do bring us more directly to the fundamental problem of how a gene can determine the production of a specific character. They possess an advantage over most traits for a study of this problem in that all stages in the development of such a trait can be observed directly. A merely descriptive study of development is evidently very far from an adequate analysis of the process but even such a meagre basis of fact is worthy of careful scrutiny in a field where all is still so obscure. Investigation of the development of a structure with a given shape in material where this shape has been analyzed genetically is therefore of value in offering a possible clue as to the method of genetic control.

The development of fruit shape provides exceptionally favorable material for studies of this sort since the entire course from the minute primordium of the ovary to the mature fruit, many thousands of times as large, may in most cases be readily followed, and since in a number of genera fruit shape has been subjected to a rather thorough genetic analy-

sis. The senior author (1929) has already described the developmental changes in various types of *Cucurbita Pepo*. In this species the wide diversity in shape in the mature fruits of different varieties is already evident in very small ovary primordia. The very elongate forms, the spherical ones, and the flattened or disk types are distinguishable as such in the youngest stages which can be measured.

Much more material of this species has recently been studied and has been analyzed according to the method suggested by Huxley (1932) and others, which eliminates the disadvantages of employing a ratio or index of dimensions as a measure of shape characters. Instead of comparing the actual size of the two dimensions studied at successive periods or determining their relative growth rate in terms of *actual* growth, this method ascertains their relative growth rate more accurately by using proportional increase (increment divided by size) as a measure of growth. Thus, essentially, the "compound interest" rates at which the two dimensions are growing are compared. This can readily be done by plotting the dimensions studied (commonly length and width) logarithmically. Their relation to each other and any changes in it may now be ascertained independently of the actual size of either.

In a growing structure the character of the line thus formed indicates very simply any alterations which may occur in relative growth in the two dimensions and thus in the shape of the structures produced. If there is no change during development this line will be straight and will have a slope (as normally plotted) of 45° . If there is any consistent and progressive change in this relationship, the line will also be straight but will have a greater or lesser slope depending on which dimension is growing faster relative to the other.

Huxley has presented a formula for the determination of this relative growth rate and thus of the slope of the line when dimensions are plotted logarithmically. If y is one dimension (length, let us say) and x the other (width), then the relation between them at any point is $y = bx^k$, where b is a constant, the initial difference between length and width (or the value of y when $x=1$), and where k , another constant, is the ratio between the growth rate (proportional or "interest" rate) of length divided by that of width. Where length and width are increasing at the same rate, $k=1$. If length is growing faster than width, k will be greater than 1 and (if length is plotted vertically) the line will slope upward more steeply; if width is growing the faster, k will be less than 1 and the line will be more nearly horizontal. Differences in the relations between length and width (and thus in shape) may therefore be due to differences in either the value of b or the value of k .

When measurements were made in *Cucurbita* of the length (polar diameter) and width (equatorial diameter) of ovary primordia and fruit

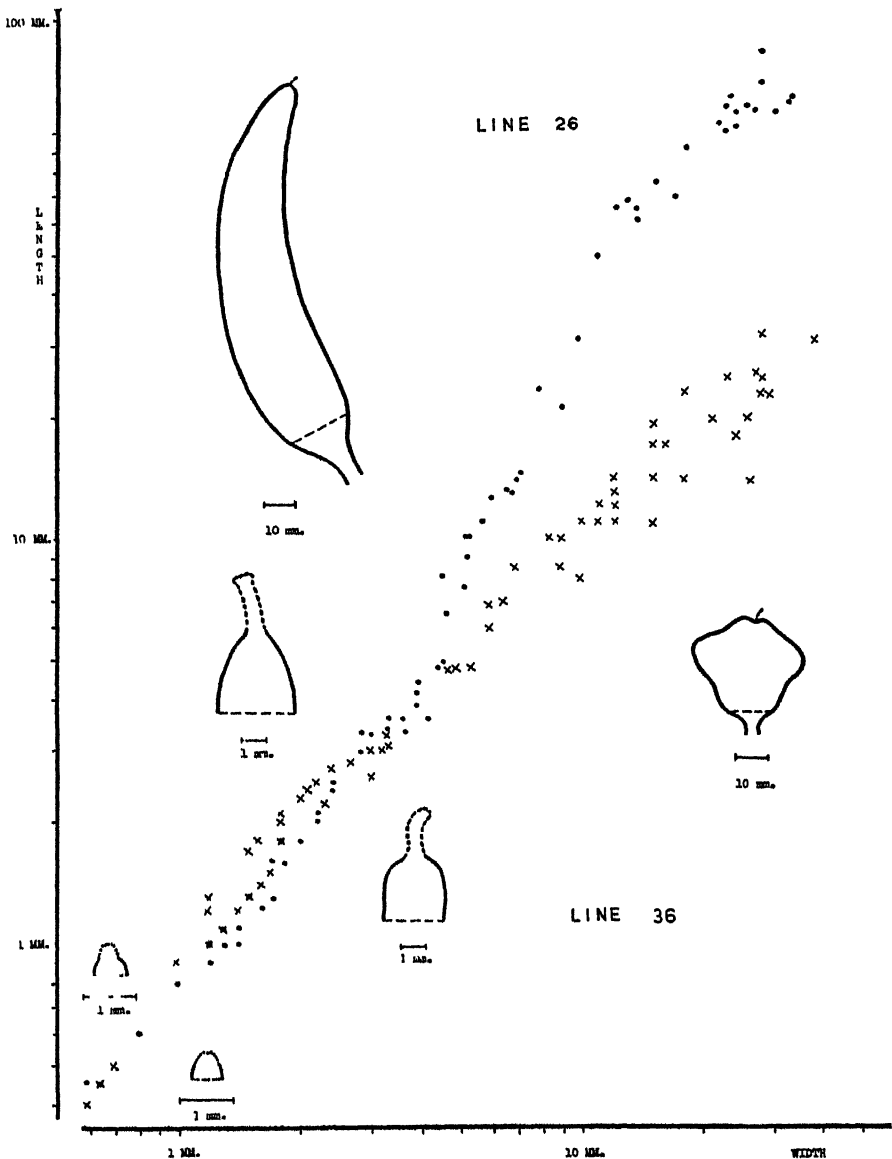


Fig. 1.

in all stages from the earliest ones observable until maturity in three lines differing markedly in fruit shape, and when each measurement was plotted logarithmically, the results shown in figure 1 appeared. Line 6 has a much

elongated fruit, line 103 an essentially spherical one, and in line 125 width is much greater than length, producing a flattened or disk fruit. The appearance in longitudinal section of young floral primordia and of mature fruit is shown in the diagrammatic sketches appended. The observations recorded are not, of course, from any single fruit but from many on several plants from the same pure line, which are thus identical genetically.

It will be noted that when length is plotted against width (both logarithmically) the result is an essentially straight line for each type, and that these lines are almost parallel, although at markedly different levels. It is also evident that the slope is a little less than $k=1$, indicating that during development width is growing at a somewhat faster rate than length and that shape is thus becoming somewhat more flattened, a fact which is evident from a comparison of the figures for primordia and for ripe fruit. The important fact, however, is that these lines *do not converge*, and that the differences between the three types in the earliest stages at which observations can be made are almost as marked as they are when the fruit is mature and has reached a volume more than a million times as great. When the tiny meristematic mass of cells which is to produce a flower has become sufficiently differentiated so that its parts can be distinguished at all, the ovary primordium has already assumed its characteristic shape.

The genetic constitution for fruit shape in these types has been determined and is relatively simple. Between lines 103 and 125, for example, there is a difference of but a single gene. We must conclude that here the shape genes exert their major influence very early indeed and that development after this earliest stage is mere increase in bulk on a pattern and at a relative dimensional rate already laid down. In Huxley's terminology, the differences in fruit shape between these lines of *Cucurbita* are in the values of b and not in the values of k .

The situation in *Capsicum* is strikingly different. In this genus the variety in fruit shape is almost as great as in *Cucurbita*. Some types have very slender, elongate fruits; others round and cherry-like ones, and in a few width is considerably greater than length. Results obtained by the junior author (unpublished) indicate that these shape differences may be analyzed genetically much as they have been in other species, but his studies of their ontogeny show that the development of shape differences here is radically different from that described above for *Cucurbita*.

In figure 2 a comparison is presented between two types of *Capsicum* fruit. Line 26 has, at maturity, a very elongate, pointed fruit. In line 36 width and length are approximately equal, though the fruit is not spherical because of the presence of an equatorial ridge. Marked as are the

differences between these two fruit types at maturity, they are almost absent at the time of flowering. The middle diagrams for each line represent the longitudinal profile of the ovary at anthesis. Except for a somewhat broader "shoulder" in line 36 they are much alike. The ratio of length to width is practically the same. In earlier primordia the resemblance is still more clear.

When length and width for the early developmental stages in these two types are plotted logarithmically, each presents an essentially straight

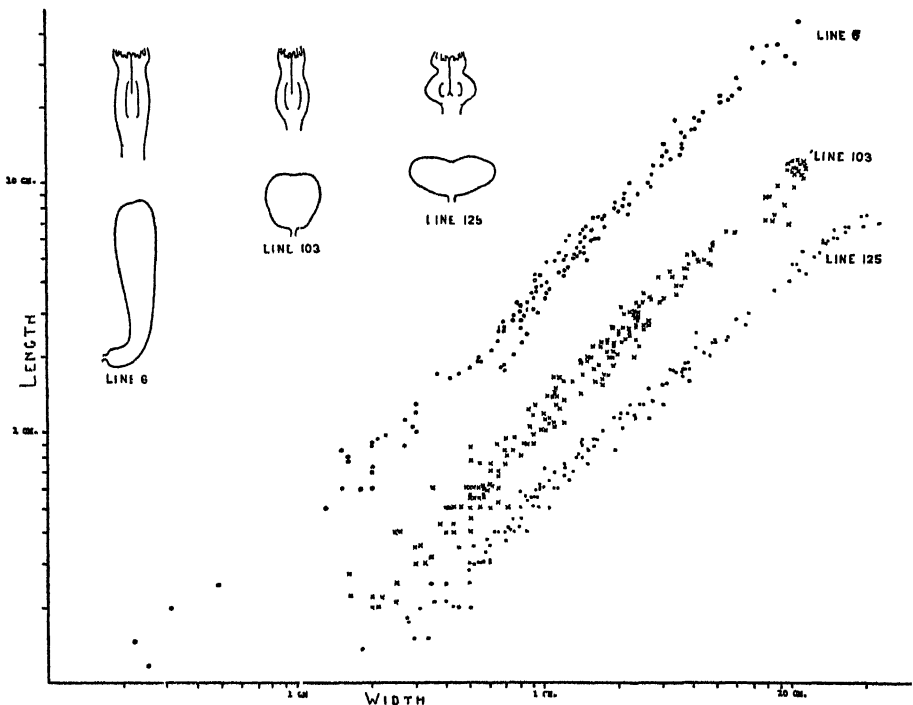


Fig. 2.

line with a value for k of a little greater than 1, and the two lines are superposed. Shortly after anthesis, however, a marked difference between the two begins to appear. In line 36 the relative growth of length and width maintains itself unchanged so that the line continues with approximately the same slope established in the early stages of development. In line 26, on the other hand, the line slopes upward steeply and abruptly, indicating the length has now begun to grow at a considerably faster rate than width. It should be understood that this difference is not only in actual rate but in true or proportional rate, as though length were now growing at a higher "rate of interest" than width. As a result of this difference the

elongate fruit shape begins to emerge. Shortly before maturity, relative length growth drops again and the slope of the line is once more not far from $k=1$.

It is evident that in *Capsicum* the major effects of the differences in genetic constitution for fruit shape are not produced very early in development, as is the case in *Cucurbita*, but appear only after a relatively late stage has been reached. Certain minor differences in outline are evident even before anthesis, but in all of the widely diverse varieties which have been studied, the ovaries during early development and for a time after flowering are essentially similar in shape. When a certain critical point is reached, the relative growth rate of the two dimensions changes rather abruptly in certain of the varieties and the differences in fruit shape so evident at maturity thus develop. The actual ratio of length to width in most types must obviously change markedly with the size of the fruit, and a determination of the "normal" or "typical" fruit shape becomes involved with problems of size and is much more difficult to determine than in the case of *Cucurbita*. Genes for shape are undoubtedly operative in both genera, but in *Capsicum* their major effect is produced late in development and is concerned with differences in the relative growth rate of the various dimensions (the value of k), whereas in *Cucurbita* it occurs very early indeed (establishing differences in the value of b) and in later development there arise no important changes in the relative growth rates which are thus inaugurated. Perhaps the early effect in *Cucurbita* is a brief but intensive alteration in the k factor. More probably it is the establishment of a fundamental pattern for growth which involves more than differences in rate alone.

The fact of chief importance which such a comparison as the present one reveals is that genes for shape may exercise their control in very different ways. In one of the types here studied they impinge early upon the developmental cycle and set up different levels of relative growth which are thereafter maintained with little modification. In the other, they seem to impinge at a much later stage and to produce their effect through a modification at that time of the growth rate of one dimension relative to that of the other. Of course in both cases shape genes must be present from the very beginning of development, and they may well be active in performing their critical rôles long before the effects of this activity are externally evident. The fact that there are such marked differences in the time of visible gene effect, however, suggests that a more detailed analysis of such divergent types may throw some light on the general problem of the relation between genetic constitution and developmental processes.

SUMMARY

1. Developmental stages from the youngest observable ovary primordium to mature fruit were studied in pure lines, differing in fruit shape, of *Cucurbita* and *Capsicum*. Length and width were both plotted logarithmically for these stages.

2. In *Cucurbita* the various shape types are visible in the earliest primordia and the relative dimensional growth rates there established persist essentially unchanged to maturity.

3. In *Capsicum* the developing ovary from its early stages until about the time of fertilization is essentially similar in shape in all types studied. Soon after anthesis, differences in the relative dimensional growth rates appear, as a result of which the shape differences which distinguish the mature fruits are produced.

4. The stage of development at which the genes for fruit shape produce their visible effect thus differ markedly in these two genera. In the terminology of Huxley, shape differences in *Cucurbita* are due to differences in the value of b ; those in *Capsicum*, in the value of k .

5. It is suggested that a study of such contrasting types may provide results of value in the problem of the relation between genetic constitution and developmental processes.

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The archegonia of *Pellaea viridis* (Forsk.) Prantl

W. N. SRENI.

When the writer (1915) reported apogamy in *Pellaea viridis*, he stated that archegonia were observed to develop on about fifty per cent of the prothallia. These sex-organs never exceeded four in number on any of the many prothallia examined. The archegonia appeared normal in every respect, and when they opened, attracted the antherozoids in large numbers. As a result of an examination of stained sections of the archegonia, no abnormal development of the egg was observed. Whether embryos develop as a result of fertilization has not been determined.

The archegonia in *Pellaea viridis* are produced, as is usual in the ordinary homosporous leptosporangiate ferns, back of the apical notch and on the well developed cushion. In this region the embryo of apogamous origin develops.

When the prothallia of *Pellaea viridis* were grown in subdued light, plates of irregular form usually consisting of a single layer of cells, and branching filaments were produced in large numbers.

In the absence of typical heart-shaped prothallia, characteristic of this species of fern, apparently normal archegonia were produced on plates and filaments which were formed in the subdued illumination.

Figure 1 represents a prothallium of *Pellaea viridis* grown for a short time in subdued light. One of the wings of the gametophyte, which had already assumed a heart-shaped form when grown in favorable light, produced in the weaker illumination a narrow plate one cell layer in thickness from which later a filament of a single layer of cells was produced. It will be observed that the filamentous portion formed a plate again, a part of which became considerably thickened. On this portion two archegonia developed. In this region the cells are considerably smaller than those in other portions of the gametophyte. The presence of small cells and the short filament of five cells, originating from this meristematic region, are evidences for the fact that an embryo of apogamous origin is beginning its development. Toward the growing end of the gametophyte, have been produced numerous short cells the walls of which for the most part are approximately parallel to one another. These cells, it is obvious, are not typically gametophytic. Such filaments have been frequently observed in the gametophyte of other apogamous ferns. In fact, cells of this nature are of common occurrence in the tongue-like outgrowth of the apical notch of the gametophyte of some apogamous ferns.

Archegonia were sometimes observed to develop on filaments consist-

ing of a single layer of cells (fig. 2). It is observed in this instance that there is a sharp line of demarcation between the cells of the filament and those of the plate.

The archegonia were frequently produced on plates composed of a single layer of cells (fig. 3). The gametophyte in this instance has also formed a filamentous portion composed of peculiar cells of regular form and which are usually associated with an embryo of apogamous origin. This portion, however, of the gametophyte is removed some distance from the archegonia.

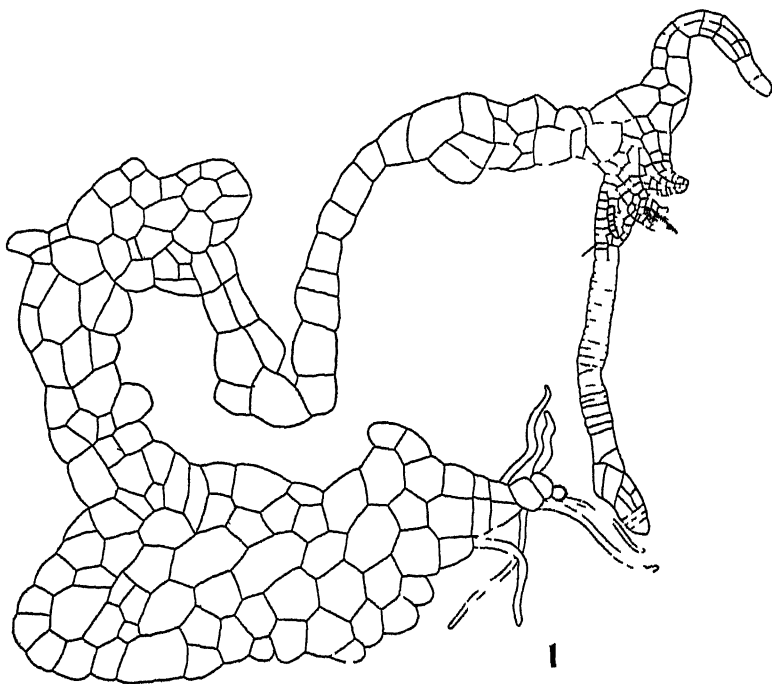


Fig. 1. A gametophyte of *Pellaea viridis* from one of the wings of which plates and filaments have been alternately formed. Archegonia and an early stage in the development of the apogamous embryo shown.

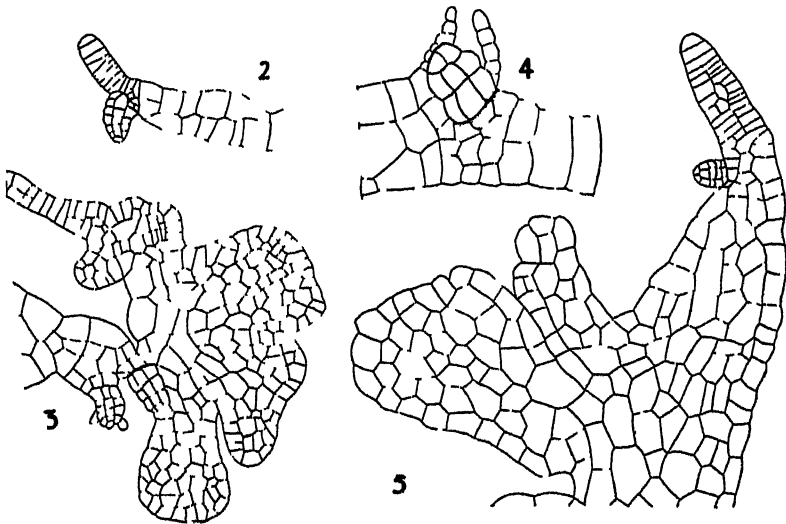
In some instances the archegonia were produced on thickened portions of plates, as is shown in figure 4. Their development was not associated in this case with an embryo of apogamous origin.

Archegonia were observed also to develop on the lobes or wings of the prothallium (fig. 5). In some instances, however, archegonia were associated with an embryo produced apogamously.

These observations made on the development of the archegonia of *Pellaea viridis* are of interest since the archegonia in the ordinary homo-

sporous leptosporangiate ferns are produced on the cushion of the gametophyte and where antheridia are not commonly formed. The writer (1919) however has reported in *Pteris ensiformis* var. *Victoria* the presence of numerous antheridia on the cushion. They completely surround the archegonia.

In the *Polypodiaceae* no one has previously reported, so far as the writer is aware, the development of archegonia on plates and filaments of the gametophyte and which have been described in species of *Trichomanes* by Bower (1888) and Goebel (1898-1901), in *Hymenophyllum* by Prantl (1875) and Sadebeck (1889), and in *Schizaea* by Britton and Taylor (1901) and by Thomas (1902).



Figs. 2-5. *Pellaea viridis*. 2. A portion of the gametophyte showing the formation of archegonia between a plate and a filament. 3. A portion of a gametophytic plate composed of a single layer of cells and on which archegonia have been produced. 4. Archegonia produced on a cushion like portion of a gametophytic plate. 5. Archegonia produced on the lobe or wing of the gametophyte.

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Multiciliate zoospores in *Physoderma Zeae-maydis*

ELIZABETH OJERHOLM

The zoospores of *Physoderma Zeae-maydis*, a chytrid which causes serious damage to corn, have been described by Shröter (1889), Tisdale (1921), and others as being universally uniciliate. In the course of a cytological study of this organism during the past year, however, occasional biciliate and triciliate swarmspores were observed. In view of the bearing which such structures have on the problem of sexuality among the Chytridiales a more thorough investigation of the zoospores was undertaken to determine the origin of this multiciliate condition and its relation to sexual fusion.

As has been shown by Tisdale (1921) and others the resting sporangia germinate readily in hanging drop cultures under optimum light and temperature conditions. During the present study they germinated in distilled and tap water within 30 to 48 hours and produced an abundance of zoospores which could thus be studied readily under the oil immersion lens. For study of the nuclear condition and behavior, zoospores were transferred in a film of water to a slide smeared with egg albumen, fixed by inverting over a bottle of osmic acid, allowed to dry and then stained in the Flemming's triple and iron haematoxylin stains. Sporangia were also planted in water in small pockets on agar plates, and as soon as germination occurred these pockets were sealed with melted agar, allowed to solidify, and cut out and fixed in Flemming's and Allen and Wilson's modification of Bouin's solution.

The zoospores of *Physoderma Zeae-maydis* are liberated from the sporangium in rapid succession one after another or sometimes in small groups through an opening in the endosporangium wall. Approximately thirty have been counted in single sporangia, but because of their rapid exit and hyaline color, it is difficult to make an accurate count. When first liberated the zoospores swim away with a jerky motion, darting about among the sporangia; but gradually movement becomes more uniform, and they swim with a swift gliding motion. Ordinarily, the zoospores move swiftly from the field of observation in a direct line, but they may also frequently come to rest abruptly and then dart off rapidly in another direction. In rare instances the entire mass of zoospores has been found to come to rest at the mouth of the sporangium and soon degenerate without any motile stage. Whether this condition is due to immaturity or lack of cilia is not yet certain.

The uniciliate zoospores vary considerably in size and shape. While

active they are usually ellipsoid in shape, somewhat pointed at the anterior end, and vary from $3 \times 4\mu$ to $5 \times 7\mu$. The cilium is attached at the posterior end and is usually four to five times the length of the spore body. In the center of the zoospore is a prominent refractive body which often appears to lie in a vacuole with one or more smaller granules. This globule has been frequently observed to shift in position when the swarmspore is in rapid motion. Quite often it protrudes somewhat at one side, as has been described and figured by Tisdale. When xylol is drawn under the cover slip the refractive globule gradually disappears and leaves behind an indefinite granular mass. A similar change occurs by treatment with ether and chloroform, and in osmic acid the globule turns black. In preparations fixed in the ordinary solutions containing acetic acid it is lacking altogether, and a large vacuole is usually present in the space it formerly occupied. The reaction of the refractive globule to these various reagents thus indicates its fatty or lipoidal nature.

According to my observations the period of motility lasts for several hours, but I am yet uncertain as to the approximate time limit. In many hanging drop cultures motility has lasted for twenty-four hours, but this may be due to delayed germination of certain sporangia. All sporangia do not germinate at the same time, and it is highly probable that the continued motility in hanging drop cultures noted above is due to this fact. Isolation and germination of single sporangia for the solution of this question has not yet been made. During the motile period the zoospores often exhibit amoeboid movements, assuming various shapes. They may put out pseudopodia at the anterior end and sides and creep slowly with the cilium trailing behind. Often, however, they may swim rapidly away with the projecting pseudopods. Quite frequently they may become attached to the substratum by their pseudopods, and in such cases they may break loose by a vigorous shaking movement and dart rapidly away. Eventually the zoospores become more sluggish in their movement, somewhat spherical in shape, and lose their cilia. Often before coming to rest, however, they may swim around in circles and revolve on their own axes.

Frequently, as has been noted before, biciliate zoospores were observed swimming among the uniciliate forms. These are usually twice the size of the latter and have two cilia of approximately the same length attached at the posterior end. In some cases they appear to originate at the same point, while in others they are slightly separated. Thousands of zoospores have been studied in the living condition, but fusion between uniciliate individuals has not yet been observed. Two somewhat amoeboid-shaped individuals have frequently been seen to come in contact, undergo further amoeboid movements, and move off together but eventually sepa-

rating. Even in the actively motile condition two zoospores may come in contact, become entangled with cilia and swim around in this manner, but in no instance has actual fusion been observed.

In fixed and stained preparations approximately one per cent of the zoospores are biciliate. They usually contain two nuclei lying in close proximity to one another, but several individuals have been found with a single nucleus, approximately twice normal size. It seems thus that this nucleus may probably have arisen from the fusion of two nuclei. Between and somewhat above the nuclei is a large vacuole, apparently the region occupied by the refractive body in the living condition. Somewhat cone-shaped regions of dense fibrous cytoplasm extend from the nuclei to the points on the periphery of the zoospore where the cilia are attached. In this region lie several small granules. The structure of these zoospores is very similar to those of the Blastocladiaceae described and figured by Kniep (1929) and Barret (1912). In most fixed and stained preparations a large number of zoospores were found lying side by side and also overlapping to the extent that no definite boundary could be seen separating them, and at first sight it appears as if numerous stages in the process of fusion were present. However, in view of the fact that fusion has not yet been observed in living material it is perhaps premature to interpret these stages as such.

However, one case of fusion between a biciliate and uniciliate zoospore has been seen. The zygote thus formed subsequently exhibited numerous amoeboid movements for approximately an hour. The cilia remained separate and intact during the various changes in form, disappearing only after the zygote rounded up and came to rest. Several triciliate zoospores have also been found in fixed and stained preparations. A similar case of fusion between a large biciliate zoospore and a smaller one has been described by Wilson (1920) in *Urophlyctis alfaiae*, which he interprets as evidence of heterogamy. Sufficient evidence, however, has not yet been found to warrant such a conclusion for *Physoderma Zeae-maydis*. Copulation between motile gametes dissimilar in size and shape has also been described for *Allomyces javanicus* of the Blastocladiaceae by Kniep (1929).

As to the origin of the tri- and biciliate zoospores described above in *Physoderma Zeae-maydis* there are, it seems to me, in general two possibilities: (1) fusion between zoospores or gametes and (2) abnormal, unequal or incomplete cleavage. Although actual fusion has been seen only in one instance, the first probability is, nonetheless, supported by evidence from other chytrid species. Fusion of zoospores or motile isogametes has been reported by Sorokin (1874) for *Tetrachytrium*, Fisch (1884) for *Reesia* and *Chytridium mesocarpi*, Woronin (1878) for *Olpidium brassicae*, Dangeard

(1889) and Chatton and Brodsky (1909) for *Sphaerita*, Lowenthal (1905), Kusano (1928, 1930) and Curtis (1921) for *Synchytrium*, Doflein (1907) for *Nucleophaga*, Kusano (1912) for *Olpidium viceae*, Wilson (1920) for *Urophlyctis*, Couch (1931) for *Micromyces zygogonii*, Swartz and Cook (1928) for *Olpidium radicale*. Köhler shows fusion between two uniciliate gametes and also between three uniciliate gametes in *Synchytrium endobioticum*, thus forming bi- and triciliate zygotes. Such fusion between three gametes has also been described among the algae by Geitler (1931) for *Tetraspora lubrica*. Among the Blastocladiaceae fusion of motile gametes has been reported by Kniep (1929) for *Allomyces javanicus*. Fusion has also been reported to occur in the parasitic slime moulds by Cook (1931) for *Sorodiscus radicolus*.

On the other hand, the view that the multiciliate zoospores are abnormal and have arisen through unequal and incomplete cleavage is likewise supported by evidence in the literature. In *Olpidium viceae* Kusano (1912) found abnormal multiciliate zoospores which he interpreted as having originated from incomplete cleavage. Lagerheim (1889) likewise figures abnormal bi- and triciliate zoospores in *Olpidicella uredinis*. In the algae also, large four ciliate zoospores resulting from incomplete cleavage have been reported by Wille (1887) for *Trentepohlia umbrina*. Going to the Oomycetes, zoospores with one, two, and three cilia have been described for *Pythiopsis* by Coker (1914). In *Isoachlya unispora* Coker (1923) figures double spores with four cilia and also masses of protoplasm which have escaped from the sporangium without forming into spores. Cotner (1930) also figures four-ciliate giant spores in *Saprolegnia monoica* var. *glomerata*. Since *Saprolegnia* and *Pythiopsis* are definitely heterogamous with non-motile antheridia and oogonia these spores have no significance from the standpoint of sexual fusion, and are doubtless abnormal. According to Cotner zoospore formation is dependent to a great degree on temperature, and the giant zoospores in *Saprolegnia* are formed as the result of unfavorable temperature conditions. Kniep also finds giant triciliate zoospores in *Allomyces javanicus* which are formed as a result of incomplete cleavage. This form then, supports both contentions as to the origin of the multiciliate zoospores. In view of this data the multiciliate zoospores observed in *Physoderma Zeae-maydis* may possibly be the result of incomplete cleavage due to unfavorable temperature conditions, since no attempt has been made in this study to control the temperature.

There is thus evidence in the literature supporting both of these viewpoints, and until further data are at hand, it would be premature to say whether or not the multiciliate zoospores observed in *Physoderma Zeae-maydis* are entirely the result of fusion or incomplete cleavage. A further

study of this problem is now in progress and will be reported in another paper on the cytology of this parasite.

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The rate of growth of the ponderosa pine in Estes Park, Colorado

OMER E. SPERRY

A study of the ponderosa pine, formerly known as western yellow pine (10), *Pinus ponderosa* Dougl., and its varieties, in its range from north to south and from east to west would lead one from British Columbia to lower California and northern Mexico, and from the Pacific coast to central Nebraska. Within this range *Pinus ponderosa* is found on fertile, moist mountain slopes, on dry rocky ridges in the midst of granite and limestone outcrops and cliffs, in fertile valleys, and on dry arid slopes of the mountains and foothills. The highest outposts are found above 9000 feet of elevation in the central Rocky Mountain region and the lowest ranges are in the northern Sierra Nevada of California and the river valleys and canyons of Nebraska at about 2500 feet. Its rate of growth in a range as great as this presents many extremes under great variations of light, temperature, precipitation, and soil moisture.

Average growth rates have been determined by comparing and contrasting trees growing in stations which present conditions of similar and comparative nature. It is the purpose of this paper to present findings as to growth rate of *Pinus ponderosa* within the area studied. The effects of mistletoe infection, of lightning damage, and observations of bark beetle injury are also noted in their relation to growth rate of the ponderosa pine.

The data were obtained within a radius of ten miles of the village of Estes Park, Colorado, except one extreme which led down the Big Thompson canyon to within a few miles of Loveland, Colorado. This places one in the eastern division of the central Rocky Mountains and is considered by some as a transition zone from north to south. With this as a location the rates of growth presented can favorably be compared with those found in mountainous regions north, west and south and with the plains or valley regions to the east.

The ponderosa pine was found growing in this area at elevations ranging from 5200 feet to 9700 feet. These, however, are the extremes and the usual range is from 6000 feet to 8500 feet. Within this range of elevation the species is found in purest stands on the south slopes. This indicates the well known fact that the ponderosa pine is capable of growing in warmer and drier situations than the other conifers of the same range.

As one goes from the south to the west slope more and more Douglas fir, *Pseudotsuga taxifolia*, is found, until on the north slope one often finds an almost pure stand of this species. From south to east this transition is more rapid than from south to west due to a more abundant moisture supply on the east slopes.

The virgin stands of ponderosa pine are very uneven-aged due to the natural succession (11). The species, growing as it does primarily in the arid portions of the area, is free from competition with other species on account of its extreme intolerance. Thus, the seedlings become established in the openings as they occur by the death or removal of the older trees and ponderosa pine follows ponderosa pine. Other species may enter but not as permanent invaders and the ponderosa pine is free to succeed itself. Aspen groves are found locally in moist sites but these are small in area, the trees are few and usually retarded and so cannot be considered important as a stage of succession. When the ponderosa pine is found growing in mixed stands, it seems to hold its own or in some cases to increase its percentage to a limited degree (5).

METHODS

The data were obtained from ten localities within the above area. All trees were measured in two localities, one a typical south slope, and one a typical west slope; while in the other localities type trees were selected at random merely as average specimens. The following data were recorded for each tree:

(1) Height. This was obtained in feet by the use of the Faustman hypsometer.

(2) Diameter. This was determined mathematically by measuring the circumference with a steel tape. All diameter measurements were taken in inches breast high, approximately four and one-half feet from the ground.

(3) Growth. Cores were taken from each tree by use of the Swedish increment borer. In all cases the cores were taken from north and south radii at the same height as the diameter measurement. The most important data were secured from these cores. By the use of a binocular microscope the annual rings were marked off in ten year periods. These periods were then measured in millimeters and recorded on work sheets for further comparisons. A total of 236 trees were included in securing data.

(4) Altitude. In order to locate extremes and to make more definite comparisons the altitudes were recorded at the various stations by use of an aneroid barometer.

(5) The type and general appearance of each tree was noted.

(6) The general habitat for each tree or group of trees was recorded in field notes.

STATIONS

Since the ponderosa pine is found in purest stands on the south slopes, the most intensive observations were carried out on these typical areas.

A slope of virgin forests on Olympus Heights, which extends from Mt. Olympus, is typical of the ponderosa pine forests of the area. The elevation of this station ranges from 7525 feet at the base to 7950 feet at the top.

Although the south slope forests are often considered a pure stand of ponderosa pine, a few other species are found. On the upper part of the slope a few Douglas fir are found, but compared to the ponderosa pine, over the entire area, they are present only in a ratio of 1 to 21. Western cedars, *Juniperus monosperma*, are scattered all along the slope and many of them are able to exist in very dry situations. The sprawling juniper,

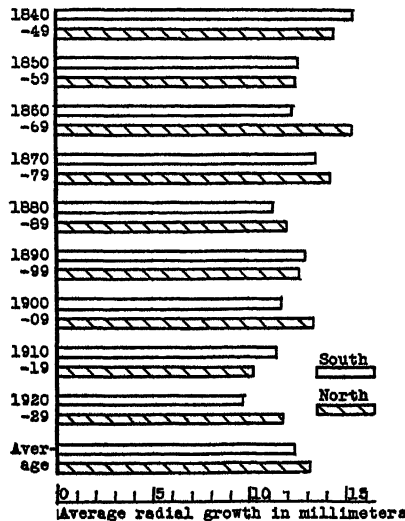


Fig. 1. A comparison of the north and the south radial growth of 95 trees under 100 years old, ring count breast high.

Juniperus communis, although not a tree, is also present. A few aspens are found, but these are small and grow only in the protected and moisture-holding coves.

The shrubs most common on this south slope area are *Purshia tridentata*, *Jamesia americana*, and one or more species of *Ribes*. Open areas 50 to 150 feet or more in diameter are often found where only shrubs and scant herbaceous growth is present. These open areas generally include much exposed rock and very shallow soil in which ponderosa pines have failed to become established.

After careful observations, a typical west slope was selected about a mile from the south slope station. The range of elevation for this slope is from 7650 feet to 8125 feet. In addition to the ponderosa pines an average of 82 douglas fir were found to the acre in the west slope station. The total

average, including a few limber pines, *Pinus flexilis*, and the junipers, was 180 trees to the acre. In contrast to this, only 106 trees were found to the average acre on the south slope. As one observes the transition from a south to a west or east slope, the change from an open to a more dense forest type is readily noted. This transition continues in this marked degree until the densest forests of the lower elevations are found on the north slopes. With this marked change in number and type of trees from south to north slope there is also noted a corresponding increase in depth of soil and the amount of humus that is retained on the surface.

The fact that the purest stands of ponderosa pine are found most abundantly on the south slopes is due to the remarkable ability of the species to adjust itself to limited moisture conditions. The actual number of trees per acre is little different from that for the west slope but moisture is not sufficient for the more moisture-loving varieties, chiefly Douglas fir. The latter are able to replace the ponderosa pines on other slopes of the same elevation since evaporation, governed to a degree by prevailing wind direction, is not so great. Then, since increase in altitude usually brings an increase in rainfall, and other climatic conditions (9), chiefly temperature, are also more favorable for tree growth, the ponderosa pines are displaced on higher slopes, more frequently by the limber pine, Englemann spruce, or alpine fir.

The ponderosa pine reaches its maximum height and diameter development when soil depth combines with soil moisture to make ideal growing conditions. These are found along the streams and rivers in areas which, as a rule, are of a limited extent. That both height and diameter growth are accelerated by water supply is shown by comparing trees growing in moist favorable situations with trees growing in dry situations. To make this comparison 89 trees under 100 years of age, ring count breast high, were studied in fourteen stations based upon three classifications; moist,

TABLE 1

A comparison of diameter growth in millimeters of trees under 100 years old, breast high measurements, divided as to habitat; moist, medium, and dry

DECADES		1920-29	1910-19	1900-09	1890-99	1880-89	1870-79	1860-69	1850-59	1840-49	DECADE AVG.
REGION	NO OF TREES										
Dry	25	11.7	13.9	16.05	19.6	20.9	21.7	19.6	17.6	20.1	17.9
Medium	48	18.9	22.3	21.9	23.5	22.5	29.1	20.5	28.5	33.7	24.54
Moist	16	39.6	40.3	47.9	55.3	42.5	38.5	48.0	35.0	43.3	43.37
Average		23.4	25.5	28.62	32.2	28.63	29.77	29.37	27.03	32.37	28.61

medium and dry. Those growing along streams and rivers were considered growing in moist habitats, those of the higher dry slopes, both west and south, as dry, and those between, of a medium range. The average diameter contrast for moist and dry situations is evident enough to show a great advance in diameter growth where water is available. The trees of medium range are related, as far as diameter growth is concerned, more closely with the trees of the dry habitat. The comparative diameter average per decade as based on core measurements was 17.9 millimeters for the dry, 24.6 millimeters for the medium, and 43.4 millimeters for the moist habitats. Comparisons of diameter growth per decade in millimeters are made in table 1.

FORM AND ECCENTRIC GROWTH

It is well known that density of stand has a marked effect upon the general form of the tree. Trees growing in the open develop a broader, lower crown and generally a more symmetrical branching effect. The slope also has a marked effect upon the form of the tree. On the down slope side the branches are often lower and in many cases longer, thus, in these cases the larger per cent of the food making area of the tree is on the down side. Steepness of slope and the immediate tree environment are both modifying factors of this condition.

Light is probably the greatest contributing factor to the unsymmetrical crown. It is evident that the greatest intensity of light is from the south, thus, the responsive development of the crown in this direction. Only a per cent of the ponderosa pines have extremely unsymmetrical crowns and not all of these are heaviest on the south. Further, there seems to be a direct relation to slope as well as to light. Of 25 trees on the south slopes all of which showed one-sided crowns, 20 were most heavily branched on the south, the others showed inclination to the west and one to the east. On the west slope this proportion was not quite so high and the balance here was as much toward the downhill side (west) as toward the south. It is evident that practically all of the one-sidedness throughout the range is to the south, southwest, or west and proportionately in this order. Wind may enter as a factor but to a minor degree since the ponderosa pine habitat is more or less protected. The chief factors influencing the balance of the ponderosa pine branch-leaf arrangement are light and slope.

Associated with the unsymmetrical crown is the eccentric growth of the bole of the tree. In a major portion of the ponderosa pines of this area growth is greatest on the north side. With some of the trees one could easily assume that a direct food relation existed. Büsgen and Münch (4) however, disprove this by an experiment of defoliation and conclude that it is a

difference in tension on the different sides of the shoot. This conclusion also agrees with Behre (3), with the exception that he considers the probability of effect which a minimum and suppressed distribution of foliation could bring about.

In order to compare the general ratio that exists between the north and south radii, the increments from these respective sides were averaged and compared. As can be noted in figure 1, the total average per decade is not constant. In four decades out of the nine, the balance was to the north, in five to the south. The average for all decades was slightly to the north.

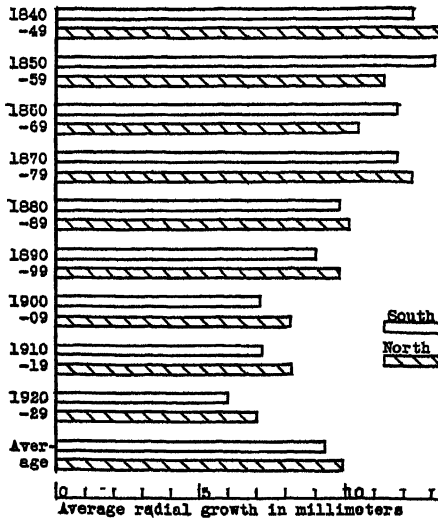


Fig. 2. A comparison of the north and south radial growth of 38 trees growing on a south slope.

This comparison is shown better in figure 2 in which 38 trees growing on a south slope were selected to compare north and south radii. The trend toward a greater north radius is more definite, thus showing that slope is an important factor. All decades except two, those in the early life of the tree, show an advance of north over south in the radial growth. The graph represents the total growth as the trees selected for this comparison were all less than 100 years old.

A numerical comparison of the north and south radial growth is made in table 2. Here also diameter comparisons are made. The minimum and maximum radial and diameter growth for individual trees per decade from 1840 to 1929 are made in this table. The minimum radial growth is found most abundantly on the south while the maximum for the north and south are about the same, averages considered. That light inhibits growth

TABLE 2
*Growth data in millimeters on trees under 100 years, total ring count
 breast high. Average 64.9 years*

DECADE	1920-29	1910-19	1900-09	1890-09	1880-89	1870 79	1860-69	1850 59	1840-49	DECADE AVE.
North Radius Maximum	31	39	39	50	30	30	32	33	21	33.9
Average	11.82	10.26	13.3	13.03	11.8	14.23	15.43	12.5	13.57	12.88
Minimum	1.15	1.4	1.2	1.5	1.5	1.5	1.6	2.5	6.3	2.1
South Radius Maximum	35	31	39	41	41	32	32	24	30	33.9
Average	9.62	11.68	11.63	13.18	11.9	13.47	12.3	12.74	17.27	12.66
Minimum	1.2	0.8	1.2	1.2	1.8	0.7	1.0	2	8	1.2
Diameter Maximum	66	70	78	91	71	62	64	57	51	67.7
Average	21.44	21.94	24.93	26.2	23.7	27.7	27.73	25.24	30.48	25.54
Minimum	2.7	2.2	2.4	2.7	3.3	2.2	2.6	4.5	14.3	2.825*
Number of trees	95	95	87	83	68	56	40	27	14	

* Omitting 1840-49.

has been shown in the case of herbaceous stems in their adjustment to light, and could be assumed in the case of woody stems. Here we have a minimum growth on the south or down slope side and also the greatest light intensity, slope and shadows being considered in the latter.

THE OPEN STAND

The open nature of the ponderosa pine stands is prominent and the three outstanding factors which contribute to this condition are light, temperature, and moisture. The species is intolerant of shade and this is effective very early in the life of the tree. The seedlings do fairly well in the shade of the parent trees, but saplings do not thrive until they receive direct light. The most important temperature relations for establishment of the seedlings are found in the surface and near-surface soil layers (2). The continued temperature requirement of the sapling and thus of the tree is that of the air, correlated with the soil surface temperature. The water requirement is relatively high as compared to the other species of the region (1). Thus, the demand of the tree for moisture leads to root

competition for soil space when moisture is insufficient. Bates (2), has shown that even though the ponderosa pine requires much heat and light, hence the warm open slopes, that it does use comparatively large amounts of water per tree of a given size. The tree is able to establish itself in its site by prompt germination and a deep, rapid, rooting habit of the seedling. Following the establishment of a deep tap root the lateral roots are extended and the severest stages of competition are brought about. The seedling is not able to compete with the roots of established trees and is consequently crowded out. Thus, the establishment and perpetuation of the open stands of ponderosa pine are controlled by temperature, light, and moisture requirements.

BIOTIC FACTORS

There are many biotic factors which have direct bearing on the rate of growth of the ponderosa pine. The two of greatest economic importance in the area are the bark beetle *Dendroctonus brevicomis* and the mistletoe *Arceuthobium cryptopodum*. Fungous parasites are also numerous throughout the region but are beyond the scope of this paper.

The bark beetle attacks trees throughout the area and many trees are observed in dead or dying condition due to its ravages. It has been estimated (7) that about 2000 beetles will actually kill a tree, but when smaller swarms attack, the tree is usually able to protect itself. The adult beetles bore through the bark to the cambium and eggs are deposited in egg galleries. They hatch very soon and the larvae spend their feeding stage in the phloem region, gradually making their way into the outer bark, pass into the pupa stage and finally emerge as adults and repeat the life cycle.

Two annual attacks are made by a brood and its offspring each year; the first in June and July, and the second in August and September. Following the second attack the brood winters in the inner bark layers of the infected tree (7). That the action of the beetle is very vigorous is evidenced by the rapid yellowing of the leaves and complete death of the tree in about three weeks. The most effective control measure is the cutting of the infected tree and removing and burning the bark in which the larvae are embedded. Some control measures of this nature are being carried out in the Estes Park area. Even though the bark beetle kills many trees, others are able to withstand the attacks and survive. These survivals can be identified by the holes left in the bark by the ingoing beetles and quite often additional holes made by birds in aiding the tree in its fight against the invader.

Hopping (6) finds that trees struck by lightning are often the target of

beetles, especially in the case of an epidemic. Miller and Patterson (8) find that bark beetle attacks are stimulated by a light burn. It is thus seen that many factors aid in bark beetle attacks throughout the ponderosa pine forest and constant control measures are essential if the pest is to be controlled.

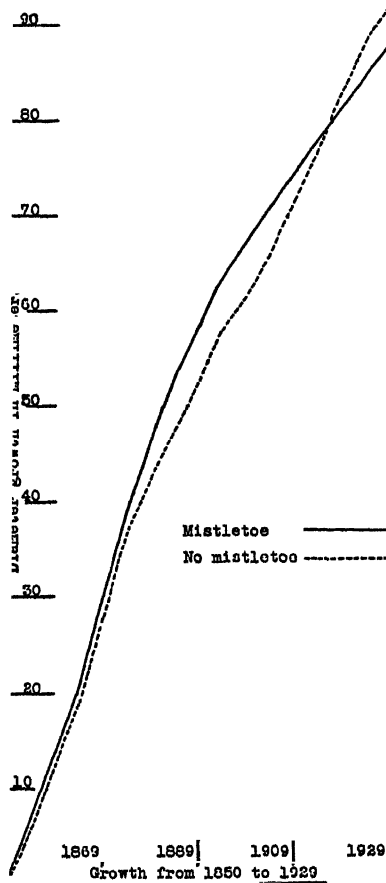


Fig. 3. To show the rate of growth of a group of trees affected by mistletoe as compared to a group free from mistletoe.

The ponderosa pine of this area is subject to severe injury by mistletoe. A tree infected by mistletoe very soon shows a decreased rate of height and diameter growth. The rate of decreased growth is directly in proportion to the severity of the infection. Accompanying this height and diameter decrease is a reduction of the leaf surface of the host. Gradually, then, the tree decreases its normal life functions and a slow death occurs (12).

The increments of five infected and five non-infected trees growing in an infected area are compared in figure 3. The trees selected were growing on the same slope in apparently similar conditions. The ages of the infected trees, according to ring count breast high, ranged from 69 to 93 years, an average of 77.4 years; the non-infected trees from 65 to 95 years, an average of 75.5 years. According to the growth curve, the infected trees were growing more vigorously than the comparative trees at the time of infection. After infection the rate of growth slowed down rapidly and in a ten year period the non-infected trees had reached the same diameter as that of the infected trees. In approximately another 15 years, at the time when the cores were taken, the average diameter of the non-infected trees was several millimeters greater and the rate of growth continuing to increase over the infected trees.

In addition to the change of rate of growth, brooms and burls are formed on the trunk of the tree. These are formed in the region of infection by using the food materials which are stored or would have been stored for the next seasons growth (12). This robs the outer branches and upper shoots of necessary food, their growth is much retarded and a striking distortion of the member is produced. As this process continues many of the outer branches die, probably the top, and possibly the entire tree.

Mistletoe is spread only by seed, either directly from the plant or carried by birds, and infection takes place in growth not more than four years old (13). The only control measures practical for an infected region is the removal of the infected member.

LIGHTNING

The effects of lightning are obvious through the ponderosa pine forest area. The most common indication of a lightning struck tree is an open scar down the trunk caused by the splitting of the bark by a current traveling down the moist, inner tissues. In some trees this scar has healed over and the remnants of it can be noted as a resinous mass in the tissues of the tree. Another effect of lightning, more obvious but not quite as common, is the "stag head." In the stag head the entire cambium region of the leader has been burned severely for a distance down the tree and as a result this portion of the tree dies. In addition to the stag head, a scar is usually formed down the trunk of the tree. In some cases the blast of the lightning bolt is enough to break off limbs or even to tear out the entire top of a tree.

Since a detailed study of the effect of lightning on an area cannot be made here, a study will be made of cores from lightning struck trees. In observing a core from the north side of a stag headed tree with no external evidence of a trunk scar, the growth ring of 1853 was found to be noticeably

narrower. Narrow rings followed for 15 years and then there was a gradual increase until the presumably normal rate of growth was reattained. On the south side annual rings could be noted after and including 1856. Inward from this for a distance of about two millimeters was a mass of resin, secreted by the tree into the lightning burn. By comparing these findings with those on the north side of the tree, it is evident that the narrow ring of 1853 was the year in which the lightning struck, and the three years difference on the south side was needed for the healing over of the wound at the place where the core was taken. The resin secretion was next to summer wood thus indicating that the lightning must have struck before growth started in the spring of 1853.

In the second type of injury, an unhealed lightning scar was noted on the south side of the trunk. The core from the north side of the tree had a very narrow ring (0.4 mm.) for the year of 1906, which was followed by larger rings (1 mm. each) for a period of four years, then normal growth was resumed up to the time the core was taken. The core from the other side of the tree had a resinous area for 1906, and a scarcely discernible ring for that year. The next season's growth showed a slight influx, then there followed six years of slow growth. From 1913 to the time the core was taken the growth was apparently normal. Thus, one can detect the time of the burn, see the immediate increase of growth in the region which enables the burn to be covered, and observe a slowing down of wood formation in the unburned region of the trunk until the burn is healed. Lightning, then, not only kills a part of the tree, causing an open wound and burn, but also slows down its normal rate of growth and causes a blemish which will be a detriment should the tree be used in the future for lumber.

TYPES OF TREES

Foresters and lumbermen divide *Pinus ponderosa* into the young, more rapidly growing trees which they term "Blackjack" and the more mature, slowly growing trees which they call the "Yellow pine." The age at which a blackjack becomes a yellow pine varies from 100 to 150 years and this in turn is modified by the environmental conditions under which the tree is growing. The blackjack type is distinguished by its darker-colored, and more deeply furrowed bark. The tree has a thicker, lower-branching crown which is often longer and more pointed than the mature type of ponderosa pine. The cork cambiums of the blackjack are narrower and longer and the bark thicker and more solid than in the mature type. This solidity is due to the thinner and more compact "borke" in the blackjack bark. The borke regions of the mature tree, being thicker and spongier, cause its outer bark to be lighter and consequently to scale off more easily than that of the blackjack.

The ponderosa pine is often termed a "rock grower." The seedlings are able to get root hold in the cracks of solid granite and secure enough moisture for continued growth. The top adjusts itself to the root spread, balance is maintained, and the tree lives.

Many rock growers of this general nature are noted whose developing roots are curtailed by the solid rock and consequently are able to secure a very limited amount of water. The top, in order to maintain a balance, has a very restricted foliage, the branches are short and contorted and many of them are dead or in a dying condition. Many of the trees in their struggle for existence have only one cluster of leaves to indicate that life is present. There are hundreds of these rock growers which are of no economic importance to the forester or lumberman but are extremely interesting to both the botanist and general observer as they live for hundreds of years with no obvious sources of materials for existence other than from the solid granite.

EXTREMES

As stated in the beginning of this paper, the ponderosa pine presents many extremes. These are partly discussed under moisture relations in that most rapid growth is found in the fertile valleys and the most retarded growth on the dry, open slopes. The elevation extremes have also been mentioned; the highest of the region was found in the south saddle of Deer mountain at an elevation of 9750 feet. The lowest elevation at which trees were observed was in the lower entrance to the Big Thompson canyon at approximately 5200 feet.

Frequently references are found stating that the ponderosa pine attains a height of over 100 feet with extremes reached in the Sierra Nevada mountains of California where heights of over 200 feet are recorded. In the area studied the tallest trees were all less than 100 feet. Of the 236 trees measured, the tallest one, growing in the Big Thompson canyon, was 92 feet high. The average height of all trees over 100 years old was 40 feet. Rock growers and young trees were not considered in the above average. These data, together with extremes and averages for age, height, and diameter are presented in table 3. Many cores were taken that had over 200 growth rings but few with over 300. Comparing these with the actual diameters and forming an estimate, it does not seem probable that many trees of the area are much over 400 years old. The greatest diameter measured was 50.9 inches and was of a tree growing in the moist valley of Fish Creek. Several other trees of this region approach this diameter and their heights range from 70 to 80 feet. Taller trees were found along the Big Thompson but their diameters were in the range of 30 inches. Other

regions of the locality probably present extremes that would equal or exceed these but to no marked degree.

TABLE 3
Averages and extremes of height, age, and diameter. Measurements breast high

		TREES UNDER 100 YEARS OLD	TREES OVER 100 YEARS OLD	ALL TREES MEASURED
Height	Maximum	60 feet	92 feet	92 feet
	Average	24.08 feet	40.34 feet	36.21 feet
	Minimum	4 feet	2 feet	2 feet
Age	Maximum	98 years	387-years	387-years
	Average	64.9 years	—	—
	Minimum	9 years	100 years	9 years
Diameter	Maximum	18 inches	50.9 inches	50.9 inches
	Average	8.7 inches	18.77 inches	13.75 inches
	Minimum	2.1 inches	4.8 inches	2.1 inches
Number of trees included		98—	138—	236

SUMMARY

A. The growth rate of the ponderosa pine is effected by many factors and the most outstanding of these for the region have been discussed. A brief summary of these is here presented.

1. The elevation at which the ponderosa pine grows ranges from 6000 feet to 8500 feet. This range is governed by moisture and temperature, especially the temperature of the soil at the time of germination and seedling establishment.

2. Slope. The ponderosa pine is found in purest, open, and uneven-aged stands on the south slopes. Mixed stands to isolated specimens are found on the west, east, and north slopes. Pure and mixed stands of limited extent are found along streams and rivers and in the valley sites. Factors governing this distribution are light, temperature, and moisture.

3. The density of the stand is in direct relation to the water supply. Light and temperature are also factors and important in the order named.

4. Growth is proportional to the water supply. This is exemplified by the comparison of equal-aged trees growing in contrasting habitats.

5. The water supply is correlated very closely with composition and quantity of soil. The valleys and lower slopes, coves and regions built up by the accumulation of humus and water-washed soil, contains much moisture, and thus afford the most ideal growing places. Trees growing in dry, barren situations are retarded according to the degree of root and

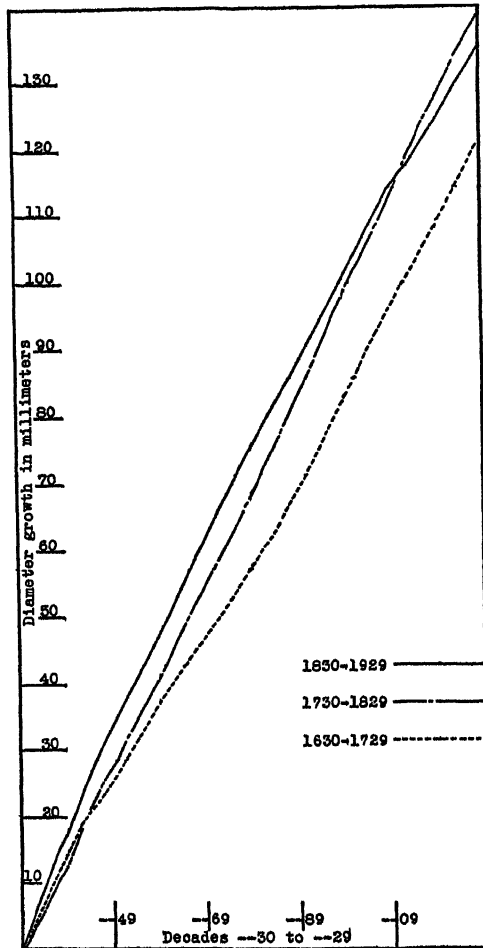


Fig. 4. A comparison of growth per decade for three centuries, 1630 to 1929.

moisture limitations. The extreme of retarded growth is reached in the rocky areas.

B. Factors more directly noted with the individual trees than with the whole area.

1. The form of the tree is modified by the density of the stand, the degree of slope on which the tree is growing, and available light. Eccentric

crown with the balance to the south or downslope side, and the eccentric growth rings with the narrow side to the south or downhill side are the two more obvious results of slope and light conditions.

2. The two chief biotic factors are the bark beetle and the mistletoe. The bark beetle attacks mature trees and either kills them or causes a marked decrease in the rate of growth until the effects of the attack are overcome. The mistletoe causes deformity of branches and bole, general slowing down in the rate of growth, and eventually the death of the tree.

3. Lightning causes "stag head" and tissue destruction by mechanical injury. A period of slow growth follows which is never entirely overcome, especially if the photosynthetic area is altered.

4. The rate of growth is the most marked during the first century of the tree's life. The rate decreases with continued growth and when the rate is materially decreased for a given tree, the summer wood becomes less distinct as compared to the spring wood.

After weighing the various factors that have direct influence upon the normal growth rate of the individual trees, of a stand, and finally of the entire area, one can readily see that any actual growth rate figures given will be very general but representative. The diameter increments of all trees measured are compared for the last three centuries in table 4.

TABLE 4
Millimeters of wood formed per decade for the last 300 years.

DECADE	1920-29	1910-19	1900-09	1890-99	1880-89	1870-79	1860-69	1850-59	1840-49	1830-39	AVERAGE
Century											
1929	10.37	10.92	12.82	13.03	11.76	14.08	13.87	13.45	15.74	18.60	14.96
1830											
1829	13.60	14.14	14.52	15.61	14.44	13.12	13.83	12.82	13.46	14.56	15.56
1730											
1729	13.56	12.21	13.88	13.04	11.47	10.10	9.25	11.53	11.27	15.00	13.57
1630											
Average											14.69

The century growth of the ponderosa pine is shown graphically in figure 4. We observe a very close correlation in the amount of wood formed during the years compared when all trees are considered. If the century 1630 to 1729 could have been represented in more trees, this correlation would undoubtedly have been closer than indicated in figure 4. There is not much variation from one decade to the next and the extremes

shown are 9.25 mm. for 1660 to 1669 and a maximum of 18.6 mm. for 1830 to 1839. These figures may have been modified by the decade group being represented in the majority by trees of a moist or dry area. The average diameter growth per decade for the last 300 years, all trees considered, is 14.69 millimeters. The average yearly diameter increment in the ponderosa pine is 1.47 millimeters.

The writer wishes to express his sincere thanks to Dr. Raymond J. Pool, chairman of the Department of Botany, the University of Nebraska, upon whose suggestion this problem was undertaken, and whose counsel was invaluable throughout its progress.¹

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¹ Contribution from the Department of Botany, University of Nebraska.

The genus *Macairea* DC. in northern South America

II. A. GLEASON

The genus *Macairea*, with twenty-eight described species, is one of the best defined and least known genera in the family Melastomataceae. The reason for the latter condition is simple: the majority of the species are found only in inland South America, especially in the savannas and mountains north of the Amazon, a vast region still inadequately explored. Of the seventeen species listed by Cogniaux forty years ago, eight were known to him through a single collection alone and three others from two collections. More material has become available since then, but more than half of the species are still represented in herbaria only by the type number. *Macairea adenostemon* DC. is well distributed in central and southern Brazil and has often been collected. There are actually more specimens of this one species in the herbaria at Kew and at Berlin than of all the other species together.

The botanical history of the genus begins with the publication of *Rhexia Radula* by Bonpland in 1820. He collected two other species also while in South America with Humboldt, but gave them no names. In 1828 De Candolle erected the genus *Macairea*, defined it exactly as understood at present, and included in it Bonpland's species and three others. The selection of a type from these four offers some little difficulty, since all of them agree well with the technical portion of the generic description. *M. Radula* has the advantage of being the only species previously named and illustrated. This and *M. adenostemon* have leaves strigose or hirsute on the upper side, while the generic description calls for glabrous leaves, such as occur in *M. rufescens* and *M. thyrsiflora*. De Candolle must have had the latter species in mind when he stated that the leaves are subvelutinous beneath, and *M. thyrsiflora* has accordingly been selected as the type. Fortunately the choice of a type is at present of no nomenclatorial significance.

Schomburgk explored British Guiana, southern Venezuela, and northern Brazil in the late thirties and collected five new species. Four of these were described by Bentham in 1840 as *M. multinervia*, *M. pachyphylla*, *M. parvifolia*, and *M. rigida*, and the fifth by Naudin in 1850 as *M. calvescens*. Spruce botanized in the upper Amazon valley in the fifties, collecting six species of *Macairea*. Two of these were already known; *M. Spruceana*, *M. sulcata*, and *M. stylosa* were described by Triana in 1871 and the sixth became the type of *M. albiflora* Cogn. in 1885. Cogniaux reviewed the genus for the Flora Brasiliensis in 1885, describing as new *M. sericea*,

M. ledifolia, and *M. Mosenii* from central and southern Brazil, and *M. albiflora* and *M. foveolata* from the upper Amazon region. This brought the total to seventeen species and no additions had been made by 1891, when his Monograph was published.

Since then eleven species have been described, based in every case on recent collections: *M. Theresiae* Cogn., 1895; *M. aspera* N. E. Br., 1901; *M. glabrescens* Pilger, 1905; *M. scabra* Cogn., 1906; *M. arirambae* Huber, 1914; *M. goyazensis* Hoehne and *M. villosa* Hoehne, 1922; *M. viscosa* Ducke, 1922; *M. duidae* Gl., *M. lanata* Gl. and *M. linearis* Gl., 1931. In almost every case these were discovered in regions previously unvisited or inadequately collected by botanists.

The treatment below does not consider six species known only from central or southern Brazil and adjacent Bolivia, *M. adenostemon* DC., *M. goyazensis* Hoehne, *M. ledifolia* Cogn., *M. Mosenii* Cogn., *M. sericea* Cogn., and *M. villosa* Hoehne. The work is based on the material in the herbaria at New York, Kew, Geneva, and Berlin, and the type or authentic material of every species but one has been examined.

Synonymy scarcely exists in the genus. One species was placed by Naudin in his heterogeneous group *Tetrameris*; De Candolle mentioned a manuscript name, which does not constitute official publication, and Bonpland described the first species known under a different genus. The remaining twenty-five species are entirely without synonyms, a condition seldom found in genera of such size.

Cogniaux' arrangement of the species is based entirely on such characters as pubescence, leaf-venation, length of the calyx, and similar features. He apparently overlooked a point in the structure of the stamens which seems to be of importance and by which two groups may be distinguished with precision. This character has been used below as the basis of the two sections of the genus.

MACAIREA DC. Prodr. 3: 109. 1828

Tetrameris Naud. in part, Ann. Sci. Nat. Bot. III. 14: 120. 1850.

Flowers 4-merous; hypanthium campanulate; calyx and sepals ascending or spreading; petals obovate, triangular-obovate, or broadly elliptic, nearly or quite symmetrical; stamens more or less dimorphic; filaments very slender, arcuate at the summit, one or both series usually glandular-pubescent on the inner side only; anthers very slender, straight or slightly arcuate, opening by a ventro-terminal pore in the short upturned beak; connective greatly prolonged below the thecae and dilated at base into a cordate, ovate, oblong, or hoof-shaped organ which is chiefly posterior, the anterior lobes minute or nearly wanting; ovary free, 4-celled (rarely 3-celled), pubescent or glandular,

at least above (said to be glabrous in one species); style slender, slightly sigmoid, often glandular below; stigma punctiform; seeds cochleate. Bushy or freely branched shrubs with short internodes; leaves usually thick or firm, comparatively small, linear to elliptic or ovate, occasionally rotund; flowers pink to purple, in small or large terminal panicles.

The connective of the short stamens is invariably stout, much shorter than the thecae, curved into a quarter or half circle, terete or shallowly channeled on the ventral side, and gradually dilated at base into a stout, fleshy, hoof-shaped organ, which is narrowly or broadly cordate in outline from the basal direction.

- I. Connective of the long stamens very slender, nearly or quite as long as the thecae, abruptly dilated at base into a flat, cordate, ovate, or oblong organ SECTION I.
- II. Connective of the long stamens essentially like that of the short stamens in shape but somewhat longer, always distinctly shorter than the thecae SECTION II.
- III. Flowers unknown

Leaves glabrous above
Leaves scabrous above

21. *M. stylosa*
22. *M. duidae*

SECTION I

Leaves glabrous above, often slightly rugose or marked with crater-like pits, usually resinous-dotted beneath.

Sepals linear.

Leaves 5-plexi-nerved; sepals shorter than the hypanthium
Leaves 3-nerved; sepals twice as long as the hypanthium.

21. *M. stylosa*.
1. *M. sulcata*.

Sepals triangular.

Strigose hairs of the hypanthium less than 0.5 mm. long; glandular hairs of the ovary very short

2. *M. thyrsiflora*.

Villous or strigose hairs of the hypanthium 1-4 mm. long; hairs of the ovary increasing distally to 1-2 mm. long.

Leaves acute, pubescent beneath with hairs, 1-1.5 mm. long; strigose hairs of the stem and hypanthium 4-5 mm. long; ovary glandular-hirsute

3. *M. rufescens*.

Leaves acute, densely resinous beneath; strigose hairs of the hypanthium 1-2 mm. long; ovary resinous and pubescent with simple hairs.

Sepals 1 mm. long; leaves 5-nerved; ovary resinous and villous in the distal half

4. *M. glabrescens*.

Sepals 2.5 mm. long; leaves 3-nerved; ovary densely strigose throughout, concealing the resin-dots

5. *M. Spruceana*.

Leaves hirsute or strigose above; pubescence of the ovary not exceeding 0.5 mm. in length, even at the tip.

Hypanthium not resinous-dotted, its longer hairs glandular.

Filaments glabrous; leaves 5-7-nerved.

Leaves acute, 5-nerved; sepals acute; petals 11 mm. long

6. *M. lanata*.

Leaves rounded above, 7-nerved; sepals obtuse; petals 6 mm long

7. *M. multinervia*.

Filaments glandular-pubescent; leaves 3-nerved

8. *M. parvifolia*.

Hypanthium copiously resinous, its longer hairs simple.

Sepals linear, much longer than the hypanthium; leaves 5-nerved

9. *M. foveolata*.

Sepals triangular, shorter than the hypanthium; leaves 3-nerved.

Leaves obtuse to rounded or retuse; connective of the larger stamens shallowly 2-lobed posteriorly.

Petals elliptic-oblong; sepals nearly 2 mm. long

10. *M. scabra*.

Petals triangular-obovate; sepals 0.7 mm. long

11. *M. arirambae*.

Leaves acute; connective of the larger stamens not lobed

12. *M. albiflora*.

SECTION II

Leaves glabrous on the upper side.

Leaves smooth above, 20-30 mm. wide

21. *M. stylosa*.

Leaves scabrous above, 2-3 mm. wide; hypanthium and ovary lepidote

13. *M. linearis*.

Leaves hirsute or strigose above.

Hypanthium and stem strigose with closely appressed, simple hairs 14. *M. aspera*.

Hypanthium glandular-pubescent with short spreading hairs; hairs of the stem spreading or curved-ascending.

Sepals broadly triangular to oblong, 2-4 mm. long; ovary 4-celled.

Filaments glandular-pubescent.

Leaves acute; petals 3.5-5 mm. long

15. *M. pachyphylla*.

Leaves obtuse; petals 7-9 mm. long

16. *M. Radula*.

Filaments glabrous; leaves obtuse, attenuate at base

17. *M. calvescens*.

Sepals narrowly triangular, 8 mm. long; ovary 4-celled

18. *M. viscosa*.

Sepals subulate, 3-4.5 mm. long; ovary 3-celled

19. *M. rigida*.

1. *MACAIREA SULCATA* Triana, Trans. Linn. Soc. Bot. **28**: 38. 1871. Known only from the type number, *Spruce 1004* (K), Tarapoto, Peru.

2. *MACAIREA THYRSIFLORA* DC. Prodr. **3**: 109. 1828. Venezuela, Esmeralda: *Tate 341* (NY); San Carlos, on the Rio Negro: *Spruce 2952* (K, B, G), *Holt & Gehriger 301* (NY); *Spruce 2953* (G); Upper Rio Negro River, Brazil: *Weiss & Schmidt* (NY); Rio Padawire, northern Brazil: *Schomburgk "no No. 5"* (K); northern Brazil: *Koch 58* (B).

3. *MACAIREA RUFESCENS* DC. *l.c.* Known only from the type collection, *Spruce 3134* (K, B, G), San Carlos on the upper Rio Negro.

4. *MACAIREA GLABRESCENS* Pilger, Verh. Bot. Ver. Brand. **47**: 165. 1905. Known only from the type collection, *Ule 6153* (B, K), from "Campina an der Ponta negra," Rio Negro, Brazil.

5. *MACAIREA SPRUCEANA* Berg apud Triana, *l.c.* Known only from the type collection, *Spruce 2013* (K, B, G, NY), from the Rio Negro, northern Brazil, between San Gabriel and Barcellos.

6. *MACAIREA LANATA* Gl. Bull. Torrey Club 58: 416. 1931. Known only from the type collection, *Tate 588* (NY) and *Tate 752* (NY), both from the summit of Mount Duida, Venezuela.

7. *MACAIREA MULTINERVIA* Benth. Hook. Jour. Bot. 2: 291. 1840. *Tetrameris trivalvis* Naud. *op. cit.* 121. 1850. Known only from the original collections of Schomburgk on Mount Roraima, British Guiana: 188.5 (type, K), 706 (K, B, G), and 1073 (K, B).

8. *MACAIREA PARVIFOLIA* Benth. *op. cit.* 293. 1840. Known only from the original collections of Schomburgk on Mount Roraima, British Guiana: 168.5 (type, K), 699 (K, G), and 1089 (K, B).

9. *MACAIREA FOVEOLATA* Cogn. Fl. Bras. 14¹: 596. 1888. Not seen by me: the type and only cited collection is *Schwacke-Glazion 9806*, from the upper Amazon valley.

10. *MACAIREA SCABRA* Cogn. Engl. Jb. 42: 133. 1908. Known only from the type collection, *Weberbauer 4708* (B), from Moyobamba, Peru.

11. *MACAIREA ARIRAMBAE* Huber, Bull. Soc. Bot. Geneve II. 6: 193. 1914. Alto Ariramba, north of Pará: *Ducke 8003* (type) and 8087 (cited by Huber). Neither of these have been examined by the author and the characters given in the present key are taken from *Ducke 10871*, from Bella Vista, on the Tapajos River, near Pará.

12. *MACAIREA ALBIFLORA* Cogn. *op. cit.* 14³: 246. 1885. Known only from the type collection, *Spruce 3719* (K), from Maypures, on the Orinoco, and from granite rocks along the Atabapo River, Venezuela.

13. *MACAIREA LINEARIS* Gl. Bull. Torrey Club 58: 417. 1931. Known only from the type, *Tate 532* (NY), from the summit of Mount Duida.

14. *MACAIREA ASPERA* N. E. Br. Trans. Linn. Soc. Bot. II. 6: 27. 1901. Slopes of Mount Roraima, British Guiana: *Quelch & Mc Connell 31* (type, K) and 24 (K); Kaictour savanna, British Guiana: *Jenman 876* (K).

15. *MACAIREA PACHYPHYLLA* Benth. *op. cit.* 292. 1840. Mount Roraima region: *Schomburgk 452* (type, K, B, G), *Quelch & Mc Connell 181* (K). *Jenman 1057* (K), 5411 (B), *Ule 8442* (B); Esmeralda, Venezuela: *Tate 323* (NY), 324 (NY).

16. *MACAIREA RADULA* (Bonpl.) DC. *l.c.* 1828. *Rhexia Radula* Bonpl. Rhex. 107, pl. 41. 1820. Known only from the type collection by Bonpland (B); the place of collection is not known but is probably along the upper Rio Negro, the Casiquiare, or the upper Orinoco.

17. *MACAIREA CALVESCENS* Naud. *op. cit.* III. 13: 35. 1850. Known only from the type collection, *Schomburgk* 521/823 (K, B, G), from the Roraima region in British Guiana.

18. *MACAIREA VISCOSA* Ducke, Arch. Jard. Bot. Rio Janciro 3: 223. 1922. Known only from the type collection, *Ducke* 2398 (K, B), from Prainha, Pará.

19. *MACAIREA RIGIDA* Benth. *op. cit.* 292. 1840. Northern Brazil or southern Venezuela, "walls of Mount Mazacca": *Schomburgk* 1015 (type, K, B, G); summit of Mount Duida: *Tate* 769 (NY).

Species imperfectly known

20. *MACAIREA THERESIAE* Cogn. Bot. Centr. 66: 369. 1896. Not seen by me; the plant was collected by Princess Therese of Bavaria along the lower Rio Negro in Brazil. Cogniaux' scanty description does not mention the stamens.

21. *MACAIREA STYLOSA* Triana, *l.c.* 1871. Along the Casiquiare River; *Spruce* 3195 (type, K, B, G); along the Orinoco River near the Atabapo; *Bonpland* (B). The chief stated character of the species is the glabrous ovary, but since the only known specimens are in fruit this feature is of doubtful value. Fortunately it may be placed in a key by foliage characters alone.

22. *MACAIREA DUIDAE* Gl. Bull. Torrey Club 58: 416. 1931. Known only from the type collection, *Tate* 1024 (NY), from the summit of Mount Duida, Venezuela. The specimen is in fruit only.

INDEX TO AMERICAN BOTANICAL LITERATURE 1931-1933

The aim of this Index is to include all current botanical literature written by Americans, published in America, or based upon American material; the word America being used in the broadest sense.

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Studies of the occurrence and transmission of virus diseases in the genus *Abutilon*

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(WITH PLATES 1-4)

Abutilon striatum Dicks. clon *Thompsonii* Veitch and its related forms which have long been cultivated because of their attractive variegated foliage, present three problems of special interest in a study of virus diseases. These are: (1) the means and methods of the transmission of the virus causing this variegation, (2) the possible immunity of green branches appearing on variegated plants, and (3) the possible occurrence of various strains or types of the virus in this group of plants.

MEANS AND METHODS OF TRANSMISSION

Abutilon Thompsonii was first displayed in 1868 by Veitch and Sons, in England, and the next year, on the continent. It was soon propagated extensively because of its attractive foliage. In propagating it Lemoine (1869) found that the variegation could be transmitted to several of the green *Abutilons* by grafting. It is probable that many variegated clons of cultivated *Abutilons* existing at the present time originated by graft infection from the clon propagated from the first variegated *Abutilon Thompsonii* plant introduced by Veitch and Sons.

Morren (1869) also found that the variegation could be transmitted not only through a variegated scion but also when a variegated leaf with its petiole was used as a scion.

Lindemuth (1902) was the first to demonstrate the transfer of the *Abutilon* variegation by intergeneric grafts among certain members of the *Malvaceae*. He succeeded in transmitting the variegation of *Abutilon Thompsonii* to *Althaea officinalis* L., *Althaea rosea* Cav., *Althaea narbonensis*, *Kitaibelia vitifolia* Willd., *Lavatera arborea* L., *Malva mauritiana* Mill., *Malva verticillata* L., *Malvastrum capense* Grcke., *Anoda hastata* Cav., *Palava malvaefolia* Cav., and *Sida napaea* Cav.

Baur (1904 and 1906) tested transmission of the *Abutilon* variegation by means other than grafting. His injections and inoculations of sap from diseased plants under normal conditions or under pressure, however, did not produce any symptoms of the variegation in normal green plants. His work was repeated and confirmed by Hertzsch (1927) and up to the present

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time no means of transmission other than by grafting are known for this virus.

It has never been shown that insects first fed on diseased and then on healthy plants of the Malvaceae are able to transmit the virus which causes variegation. This, however, does not preclude the possibility of such transmission by native insects in tropical countries where many of these plants are indigenous, but no study regarding this matter appears to have been made. Green plants have never become variegated under greenhouse conditions, although "Gardener's Chronicle" (1869, p. 276) states: "*Abutilon striatum* occasionally throws out a variegated shoot without being grafted." This report has never been substantiated.

The transmission of variegation through the seed has never been definitely observed for any of the variegated Malvaceae by previous experimenters and hence the conclusion, that there is no transmission through seeds. Morren (1869), without mentioning specific cases, says merely, "Les graines des plantes panachées donnent—des plantes saines et normales." Baur (1906) states repeatedly that "Sämlinge bunter Pflanzen sind immer wieder rein grünblättrig." He mentions specifically obtaining none but green seedlings from 300 seeds of a variegated plant of the species *Kitaibelia vitifolia* and from 70 seeds of a variegated plant of *Abutilon indicum*. He believes that the quantity of the virus present in the seeds is too small to infect the embryo.

Lindemuth (1907), reports: "Alle Samenpflanzen der durch Transplantation mit *Abutilon Thompsonii* buntblättrig gewordenen Abutilonarten und -sorten haben rein grünblättrige Nachkommen ergeben." As examples, he reports seeds obtained from *Althaea rosea*, *Malvastrum capense*, *Abutilon Avicennae* Gaertn., *Abutilon megapotaemicum* St. Hil., *Abutilon* var. Erfurter Glocke, *Kitaibelia vitifolia*, *Lavatera arborea*, *Anoda hastata*, all of which had become variegated through grafting with *Abutilon Thompsonii*. In no case does he give the exact number of seeds and seedlings obtained, merely referring to hundreds of seeds. Commercial seeds said to be of *Abutilon Thompsonii* which he bought produced only green seedlings. He also obtained seeds from *Abutilon megapotaemicum* which produced twenty seedlings, three of which showed intensely variegated leaves. Two months later, two seedlings had lost this "variegation" completely; the third one remained variegated. It is not stated whether the seeds were obtained from a variegated or from a green plant. Lindemuth claims that the variegation in the seedlings originated without being grafted. This suggests the possibility of virus transmission through seeds.

Hertzsch (1927) obtained seeds from a severely infected plant of *Abutilon indicum* but only green seedlings grew from them. His explanation

is that the embryonic tissues are not enough differentiated to be infected by the virus.

Cook (1931) reports from the West Indies, that seeds procured from variegated plants of *Abutilon hirtum* Sweet have produced none but green seedlings.

Lindemuth definitely reports seed of variegated Abutilons, obtained in all cases by cross-pollination, as follows: (1) *A. Thompsonii* \times *A. var. Rêve d'Or*, seeds produced only green seedlings; (2) *A. var. Erfurter Glocke* \times *A. Darwinii* var. *tesselatum*, seeds produced only green seedlings with small lacinate leaves; (3) *A. var. Cannell* \times *A. Thompsonii*, seeds produced some green seedlings and some with greenish-yellow spots and dots. All seedlings showed diversity of leaf shapes, resembling those of either parent or very irregular and lacinate; (4) *A. Darwinii* var. *tesselatum* \times *A. Thompsonii*, seeds produced green and yellow-flecked lacinate leaved seedlings. Thus Lindemuth's seedlings possessed yellow-flecked leaves in some cases. He calls such variegation "falsche Panaschüre." Such seedlings were never proved to be truly virus infected in so far as the transmission of their "variegation" to susceptible green plants is concerned, because no grafting tests were reported.

Thus the evidence was interpreted to indicate that the variegation found among the several clons, varieties and species of *Abutilon* is not transmissible through the seed. Although some seedlings showed yellow flecks and spots, they were considered as being different from the truly variegated types.

MATERIALS USED

The types of plants used in these experiments are the following:

Abutilon striatum clon *Thompsonii*. Propagated from a cutting of the original stock obtained in 1914 by Dr. A. B. Stout from Mr. R. L. Lynch who was then Director of the Cambridge Botanical Gardens, Cambridge, England.

Abutilon megapotaemicum variegatum. Characterized by semi-trailing habit, narrow leaves showing variegation in large blotches. One clon was obtained from the Central Park greenhouses; another clon from Mr. S. Untermyer's estate at Yonkers, New York.

Abutilon clon "Garden Stock." This clon was propagated by the New York Botanical Garden for outdoor bedding purposes and differs from the *Abutilon Thompsonii* clon in intensity of variegation (yellow and green variegation, rather than white, yellow and green variegation), and in leaf shape (more narrowly pointed lobes).

Abutilon clon "Eclipse" Hort. This clon is similar to *Abutilon mega-*

potamicum variegatum except for its more erect habit, slightly larger leaves and petal coloration. The petals are pale orange rather than lemon yellow and the flower is more open. Obtained from Vincent's Nurseries at White Marsh, Maryland. Originated probably as a seedling.

Abutilon clon "Mulleri" Hort. (or Milleri). Similar to *Abutilon* clon "Eclipse" except for irregularities in leaf shape (more lacinate). Also probably of seedling origin. Obtained from Mr. Baudish of Pleasantville, New York.

Abutilon clon hybridum. Maple shaped leaves, similar to those of *Abutilon Thompsonii*, but less pointed and more irregularly variegated (pale blotches and small spots). Obtained from horticultural plantings.

Abutilon Regnellii Miq. A green-leaved species grown from seed obtained from Brazil. Leaves entire, not maple-shaped. This species is very susceptible to transmission of the variegation by grafting, showing symptoms quickly and distinctly, and is therefore frequently used as an "indicator."

A green clon was obtained from a seedling of *Abutilon* grown by Professor Hugh Findlay. The leaves were deeply five-lobed resembling those of *Abutilon* clon "Garden Stock."

TESTS FOR TRANSMISSION OF VARIEGATION THROUGH SEED

In pollination experiments with the variegated clons studied, it was found that no seed is formed when these clons are self-, close-, or intra-clon pollinated. The bell-shaped flowers sometimes show incomplete or partial dichogamy. The numerous stamens are united at the lower end of their filaments to form a tube inside of which the gynoeccium is found. When some flowers are open, pollen is being shed from the topmost anthers, and the styles which are united below and free above are still within the staminal tube. The stigmas protrude, however, before all pollen has been shed, and in some instances the styles curve and twist so as to come in contact with the pollen-shedding anthers, effecting self-pollination. The failure to set seed to self- and close-pollination is due to self-incompatibility.

The green species *Abutilon Regnellii* forms seed readily to self-pollination, and it becomes readily and strongly variegated when grafted with variegated scions. Several plants of *Abutilon Regnellii* were made variegated through graft infection with each of the five variegated clons used in these experiments. Seeds formed readily on the stock plants, which in many instances showed infection to such an extent that even the sepals and seed capsules became variegated. The seeds, obtained after variega-

tion had become general in a plant, were sown and results of the germination are reported in the following table:—

TABLE 1
Seeds and seedlings obtained from graft infected Abutilon Regnellii stock

SCION ON A. REGNELLEI	NUMBER OF SEEDS	NO. OF SEEDLINGS GROWN	SEEDLINGS SHOWING VARIE- GATION AFTER SIX MONTHS
<i>A. Thompsonii</i>	328	275	0
<i>A. mega pot. var.</i>	191	123	0
<i>A. Eclipse</i>	236	173	0
<i>A. Mulleri</i>	210	187	0
<i>A. Gard. Stock</i>	372	211	0
Totals	1337	969	0

In these cases no variegated seedlings were obtained.

All possible combinations of cross-pollinations were made between the variegated clons. In these cases the seedlings obtained had both parents variegated. The data for these seedlings are shown in table 2.

The following table indicates that out of a total of 3915 seeds only 2229 or 57% germinated and that of the 2229 seedlings, 349 show "apparent variegation" or mottling. In all but two instances such mottling appears in some of the seedlings obtained from each inter-clon pollination of the variegated *Abutilon* clons. This "variegation" is somewhat unlike that of either parent. The variegation of the seedlings is less pronounced than that of the parents. In the latter, the green areas of the leaf are in sharp contrast to the white or yellow variegated parts, while in the "variegated" seedlings, the green areas are only indistinctly contrasted with pale green or yellowish areas. In addition, the "variegation" of seedlings obtained from the same parents differs in degree, extent and general appearance. Occasionally some seedlings show normal green leaves and branches in addition to "variegated" leaves. Such seedlings may remain in this condition or the entire plant may become green leaved eventually. In no instance have green branches become "variegated"; but "variegated" branches have infrequently produced young green leaves. This occurred mostly where the "variegation" consisted of a pale green mottling, sometimes discernible only in diffused light. Similar results were described by Dr. A. B. Stout in a paper delivered at the meeting of the A.A.A.S. in Philadelphia (1926) but at that time no tests had been made to determine if such "variegation" could be transmitted.

Table 2 also indicates that with four exceptions the progeny contained some seedlings with irregular laciniate leaves, although the great majority of the seedlings had leaves resembling the parent plants in shape. Plants with such asymmetrical laciniate leaves are usually smaller than normal

seedlings. Occasionally such plants produce branches with regular, symmetrical leaves which tend to be more vigorous. In most cases such lacinate leaves show evidences of "variegation," although the lacinate char-

TABLE 2

Data for seeds and seedlings obtained from inter-clonal pollinations of variegated clons

	SEEDS			SEEDLINGS	TOTAL
	TOTAL NUMBER	GERMINATED	GREEN	VARIEGATED	LACINATE
<i>Abutilon striatum</i> clon Thompsonii Seed Parent					
Selfed	0				
× <i>A. Eclipse</i>	207	112	97	15	11
× <i>A. Mulleri</i>	214	150	127	23	7
× <i>A. megapot. var.</i>	135	96	82	14	5
× <i>A. Garden Stock</i>	139	81	77	4	0
× <i>A. hybridum</i>	55	22	22	0	0
<i>Abutilon</i> clon Eclipse Seed Parent					
× <i>A. Thompsonii</i>	81	55	49	6	6
Selfed	0				
× <i>A. Mulleri</i>	255	124	106	18	16
× <i>A. megapot. var.</i>	335	216	177	39	10
× <i>A. Garden Stock</i>	208	103	97	6	2
<i>Abutilon</i> clon Mulleri Seed Parent					
× <i>A. Thompsonii</i>	247	159	114	45	29
× <i>A. Eclipse</i>	180	101	73	28	12
Selfed	0				
× <i>A. megapot. var.</i>	163	101	84	17	11
× <i>A. Garden Stock</i>	102	37	30	7	3
× <i>A. hybridum</i>	265	88	82	6	2
<i>Abutilon megapotamicum variegatum</i> Seed Parent					
× <i>A. Thompsonii</i>	143	98	84	14	5
× <i>A. Eclipse</i>	268	175	143	32	7
× <i>A. Mulleri</i>	213	158	121	37	12
Selfed	0				
× <i>A. Garden Stock</i>	0				
× <i>A. hybridum</i>	0				
<i>Abutilon</i> clon Garden Stock Seed Parent					
× <i>A. Thompsonii</i>	209	156	151	5	0
× <i>A. Eclipse</i>	172	88	73	15	7
× <i>A. Mulleri</i>	105	37	22	15	1
× <i>A. megapot. var.</i>	0				
Selfed	0				
× <i>A. hybridum</i>	0				
<i>Abutilon hybridum</i> Seed Parent					
× <i>A. Thompsonii</i>	12	5	5	0	0
× <i>A. Eclipse</i>	0				
× <i>A. Mulleri</i>	207	67	64	3	1
× <i>A. megapot. var.</i>	0				
× <i>A. Garden Stock</i>	0				
Selfed	0				
Totals	3915	2229	1880	349	147

acteristics themselves cannot be definitely attributed to action of the virus. Lesley (1928) observed similar leaf distortions in 4 per cent of the seedlings obtained by crossing two "healthy" tomato varieties.

When variegated scions such as *Abutilon Thompsonii* are grafted on the "variegated" seedlings, the contrast between the green and the yellow leaf parts of the latter is greatly intensified. This intensified variegation, but not the mottling, can be transmitted to susceptible green scions such as *Abutilon Regnellii*. The symptoms produced in the green scions are in no way different from those produced by grafting scions of *Abutilon Thompsonii* and others directly on *Abutilon Regnellii*.

To test the transmissibility of the laciniate condition and of the "variegation" shown by some of the seedlings, the latter were grafted on and with *Abutilon Regnellii*. In no instance were the laciniate characteristics transmitted to scion or stock of *Abutilon Regnellii* (see table 3). No transmission was noted in most other cases, even after a graft union of several years standing.

Evidence of transmission of the variegation through seed was first noted in the case of two seedlings obtained by crossing *Abutilon Mulleri* with *Abutilon Thompsonii*. Similar crossings were repeatedly made and a few additional "variegated" seedlings were again shown to be capable of transmitting their "variegation" to susceptible green Abutilons. The data are recorded in the following table.

TABLE 3
Data on seeds and seedlings of *Abutilon* clon *Mulleri* × *Abutilon* clon *Thompsonii*

TOTAL NUMBER	SEEDS		SEEDLINGS LACINIATE	VARIEGATED	VARIATION TRANSMISSIBLE
	GERMINATED	GREEN			
36	18	0	9	9	0
27	22	10	6	12	0
35	12	5	6	7	1
19	15	12	0	3	1
78	70	57	8	13	1
48	6	5	0	1	0
162	128	106	7	22	1
Total, 405	271	204	36	67	4

The 67 "variegated" seedlings were tested with scions of *Abutilon Regnellii* to determine the transmissibility of their "variegation." Of the 67 grafts that were made, 48 proved successful. In these tests only 4 seedlings were capable of infecting *Abutilon Regnellii*. One of these is shown in plate 2. The symptoms produced were milder and less pronounced than when any other variegated Abutilon was grafted either on *Abutilon Regnellii* or on the Abutilon seedling (green clon). From this evidence, it is

concluded that the virus producing variegation in *Abutilon* clon Mulleri and *Abutilon* clon Thompsonii is in a limited way transmissible to some of the seedlings obtained when they are crossed.

Seedlings were obtained from crosses between variegated and green *Abutilon* clons and the data for these are tabulated as follows:

TABLE 4

Data on seeds and seedlings obtained from inter-clon pollinations of green and variegated clons

	NUMBER OF SEEDS	GREEN	SEEDLINGS VARIEGATED	LACINIATE
Abutilon Seedling (green clon) Seed Parent				
Self-pollinated	0			
Close-pollinated	0			
Intra-clon pollinated	0			
× <i>A. Thompsonii</i>	111	62	2	2
× <i>A. Eclipse</i>	35	12	2	0
× <i>A. Mulleri</i>	101	22	11	10
× <i>A. megapota. var.</i>	16	10	0	0
× <i>A. Garden Stock</i>	44	30	1	0
× <i>A. hybridum</i>	504	185	3	0
Abutilon Seedling (green clon) Pollen Parent				
× <i>A. Thompsonii</i>	39	26	0	0
× <i>A. Eclipse</i>	48	29	2	0
× <i>A. Mulleri</i>	64	22	11	0
× <i>A. megapota. var.</i>	65	30	4	0
× <i>A. Garden Stock</i>	69	47	0	0
× <i>A. hybridum</i>	422	165	9	0

The above table shows that, for the offspring of variegated × green *Abutilons* and of green × variegated ones, of a total of 685 seedlings only 45 showed "variegated" characteristics. When 31 of these 45 "variegated" seedlings were successfully grafted on and with *Abutilon Regnellii* in no case did transmission of "variegation" take place. The laciniolate seedlings mentioned in tables 3 and 4 were incapable of transmitting this condition to normal green plants.

POSSIBLE RECOVERY AND IMMUNITY

The earlier investigators noted that *Abutilon* varieties show differences in resistance to infection by grafting. Some varieties showed no infection or they lost their infection soon after becoming variegated. The first case of this was reported by Morren (1869) for *Abutilon tonelianum* from Mexico, which showed only temporary transmission of the variegation after grafting. He did not state whether this loss of variegation indicated a recovery from the disease or whether it was due to shading or loss of variegated leaves. Nor did he further indicate whether or not this recovery made the plant immune to further graft transmission experiments. If,

without any special treatment, this plant should have lost its variegation, then we would find here the first record of an *Abutilon* recovering from the virus.

Lindemuth (1872, 1878) claimed a similar recovery for *Abutilon arborum* Sweet, *Abutilon* var. *Martius*, *Abutilon Pattersoni*, and *Abutilon vitifolium* Presl. In these cases he noticed the appearance of flecks, either large and single, or in groups scattered throughout the leaves. As the leaves grew larger, the flecks often became rarer until sometimes they entirely disappeared. Lindemuth did not perform any experiments, however, to determine whether such recovery represented permanent immunity. When biennials and herbaceous perennials such as *Althaea rosea*, *Malva mauritiana*, *Malva verticillata*, *Lavatera arborea*, *Kitaibelia vitifolia*, *Sida napaea*, have once become infected through grafting with *Abutilon Thompsonii* they remain variegated even after a winter rest. *Althaea officinalis* is an exception to the above since it loses its acquired variegation during the winter. Such plants can be reinfected, however, hence they are not permanently immune. During his work with intergeneric grafts among the Malvaceae, Lindemuth found that various genera show different degrees of susceptibility to infection by grafting, as in the case of *Lavatera arborea*, *Sida Napaea* and *Althaea officinalis*. After grafting *Abutilon Thompsonii* on various members of these three genera, he observed that some plants of each genus showed no transmission. Such apparent immunity does not necessarily indicate absolute immunity, for the appearance of the first symptoms of variegation may be greatly retarded, due to slow and irregular spread of the virus through the plant depending upon age, health and vigor of plant and scion, perfection of graft union, and finally, length of period of observation. In some instances, as shown in this paper, more than one year elapsed, after grafting, before the first symptoms of variegation appeared.

Baur (1906) confirmed Lindemuth's findings with regard to recovery after winter rest and to varying susceptibility. He also demonstrated that variegated plants could be "cured" by removing all leaves and then placing plants in the dark, and also by removing several successive crops of variegated leaves. The resulting green plants were not immune but could be reinfected.

The most conspicuous recovery from variegation is seen when a green-leaved branch develops on a variegated plant. Concerning the appearance of such green branches, Baur states, "Es kommt vor, dass auf buntblättrigen Exemplaren einzelne Zweige auftreten, die dauernd grün bleiben." He reported these in the case of *Abutilon Thompsonii*. Such a branch was propagated by cuttings and he claimed that the resulting clone was immune

to the mosaic, although ordinary *Abutilon striatum* plants are highly susceptible. When the "immune" individuals of this clon were grafted on other variegated Abutilons no transmission occurred. Baur also found, as had Lindemuth, that immune green clons of species usually susceptible were in existence. Such is the case with *Abutilon arboreum* Sweet. He demonstrated that such immune branches, while not showing any visible symptoms of the mosaic nevertheless allowed passage of the virus. *Abutilon indicum* Sweet became variegated after it had been grafted on an immune green branch of *Abutilon arboreum*, which in turn had been in graft union with *Abutilon Thompsonii*.

Price (1932) working with species of *Nicotiana* noted that plants infected with ring-spot virus produced healthy appearing leaves, resulting in complete recovery of the plants in most cases. If such plants were inoculated with ring-spot virus or grafted on or with diseased plants no symptoms of the disease were produced, indicating an acquired immunity. This immunity persisted when the plants were propagated as clons through three generations from cuttings. He found however that juice and scions obtained from all the recovered plants were highly infectious and capable of transmitting the ring-spot virus to healthy plants.

In the course of the present experiments, the observations made by Lindemuth and Baur with regard to the appearance of green branches on variegated Abutilon plants are confirmed. It was noted in the cultivation of *Abutilon megapotamicum variegatum* that entirely green leaves and even green branches are of frequent occurrence. (Plate 5) If green branches continue to be formed the entire plant may eventually become green. In the other variegated Abutilon clons used in these experiments, the appearance of green branches on individual plants was less frequent. Whenever such branches were propagated as clons the resulting plants remained green and never showed any evidence of spontaneous variegation. Efforts were made to determine the following points:

- a. Whether the virus is entirely absent or only masked in the green branches appearing on variegated plants.
- b. Whether the virus may be transmitted through such green branches to infect a susceptible scion grafted on them.
- c. Whether stimulated flow of sap will cause appearance of the variegation in green branches of variegated plants.
- d. Whether such green branches are actually immune.

Various methods of grafting were tried to test transmission. Whip grafting proved the most successful. Raffia was used in holding the scion to the stock and a paraffin coating applied over the raffia to prevent drying out. Scions used were two to three inches in length. Immediately after

grafting, plants were placed either under bell jars or in moist chambers (wooden framework with muslin covering kept moist). They were kept in these containers for two weeks or more, while light and air were admitted gradually, from day to day, until finally plants could be placed in a shaded part of greenhouse bench. The most successful grafts were made during the spring and the fall of each year. After a month or more the raffia binding was slit.

In testing for the possible presence of the virus in green branches of variegated plants, scions of such green branches were grafted on *Abutilon Regnellii* and on a green clon of an *Abutilon* seedling. At the same time reciprocal grafts were made by cutting off the tops of such green branches, and adding *Abutilon Regnellii* scions. The results of the experiments are tabulated in tables 5 and 6.

TABLE 5

Data on grafts made to indicate the presence or absence of the virus in green branches of variegated Abutilons

STOCK GREEN	SCIONS GREEN	GRAFTS	APPEARANCE OF VARIATION
<i>A. Regnellii</i>	<i>A. Thompsonii</i>	7	0
<i>A. Regnellii</i>	<i>A. Eclipse</i>	5	0
<i>A. Regnellii</i>	<i>A. Mulleri</i>	5	0
<i>A. Regnellii</i>	<i>A. megapot. var.</i>	6	0
<i>A. Regnellii</i>	<i>A. Garden Stock</i>	6	0
<i>A. green seedling</i>	<i>A. Thompsonii</i>	8	0
<i>A. green seedling</i>	<i>A. Eclipse</i>	5	0
<i>A. green seedling</i>	<i>A. Mulleri</i>	6	0
<i>A. green seedling</i>	<i>A. megapot. var.</i>	4	0
<i>A. green seedling</i>	<i>A. Garden Stock</i>	11	0

From the above, it appears that the virus is absent in green branches of variegated *Abutilons* for in no case did symptoms of the variegation appear in susceptible stock plants after grafting with green scions.

TABLE 6

Results of grafts to test the passage of the virus through green branches of variegated Abutilons

STOCK VARIEGATED WITH GREEN BRANCHES	SCION GRAFTED ON TOP OF GREEN BRANCHES	GRAFTS	APPEARANCE OF VARIATION
<i>A. Thompsonii</i>	<i>A. Regnellii</i>	4	0
<i>A. Thompsonii</i>	<i>A. green seedling</i>	5	0
<i>A. Eclipse</i>	<i>A. Regnellii</i>	3	0
<i>A. Eclipse</i>	<i>A. green seedling</i>	4	0
<i>A. Mulleri</i>	<i>A. Regnellii</i>	3	0
<i>A. Mulleri</i>	<i>A. green seedling</i>	3	0
<i>A. megapot. var.</i>	<i>A. Regnellii</i>	4	0
<i>A. megapot. var.</i>	<i>A. green seedling</i>	2	0
<i>A. Garden Stock</i>	<i>A. Regnellii</i>	5	0
<i>A. Garden Stock</i>	<i>A. green seedling</i>	3	0

The results reported in table 6 indicate that the virus does not pass through green branches present on variegated *Abutilons* in sufficient quantity to show symptoms of the variegation in susceptible green scions when these were grafted to the green branches. After the scions were successfully established, the variegated parts of the plants were trimmed down in the effort to stimulate flow of sap into the green branches. This, however, failed to produce any symptoms of the virus in the green scions.

To determine whether the green branches appearing on variegated plants are actually immune, the following test was made. Vigorous plants were obtained from cuttings of the green branches and then scions of variegated plants were grafted to them. The results are shown in table 7.

As shown in table 7, plants propagated from green branches of five different types of variegated clons were grafted with scions of all five types of variegation. None of the green plants thus tested was actually immune, since it could be reinfected. Such reinfection was accomplished not only by grafting on them variegated scions of other *Abutilon* clons, but also scions of their own variegated clon, (plate 1) providing plants and scions used were young, and not woody. When scions gradually became more woody it was noted that a longer time elapsed before symptoms indicating the transmission of the variegation appeared.

THE POSSIBLE OCCURRENCE OF STRAINS OR TYPES OF THE VIRUS

Hertzsch (1927) first recognized the existence of two different types of virus among variegated members of the *Malvaceae*. He named these types "A" and "B" chlorosis. As a basis of comparison, he uses the different symptoms produced by these types when grafted on identical plants. His "B" chlorosis produces more pronounced symptoms or a more intense variegation than the "A" type. He also finds that the species *Lavatera arborea* is immune to the "A" type and susceptible to "B."

In the course of the grafting experiments to determine immunities, it was noted that scions of different variegated clons may produce different symptoms when grafted on green plants of the same clon. Thus, when *Abutilon Thompsonii* and *Abutilon* Garden Stock are grafted on green *Abutilon Thompsonii* plants, different symptoms are produced. The variegation of *Abutilon Thompsonii* is much more intense than that of the clon Garden Stock. The same difference in intensity is evident in the transmitted symptoms when each is grafted on green plants of any of the five *Abutilon* clons used in these experiments.

When *Abutilon Thompsonii* is grafted on variegated plants of *Abutilon megapotamicum variegatum*, *Abutilon Eclipse*, and *Abutilon Mulleri*, no

TABLE 7

Results of grafts to test the possible immunity of green branches of variegated Abutilons

STOCK GREEN PLANTS	SCION VARIEGATED	GRAFTS	FIRST APPEARANCE OF VARIEGATION IN DAYS
<i>A. Thompsonii</i>	<i>A. Thompsonii</i>	2	30
		2	55
<i>A. Thompsonii</i>	<i>A. Eclipse</i>	1	70
		2	115
<i>A. Thompsonii</i>	<i>A. Mulleri</i>	2	55
		1	30
<i>A. Thompsonii</i>	<i>A. megapot. var.</i>	4	110
		1	370
<i>A. Thompsonii</i>	<i>A. Gard. Stock</i>	2	40
		2	60
<i>A. Eclipse</i>	<i>A. Thompsonii</i>	2	40
		1	40
<i>A. Eclipse</i>	<i>A. Eclipse</i>	2	60
		2	40
<i>A. Eclipse</i>	<i>A. Mulleri</i>	3	70
		1	50
<i>A. Eclipse</i>	<i>A. megapot. var.</i>	1	100
		3	90
<i>A. Eclipse</i>	<i>A. Gard. Stock</i>	1	50
		4	70
<i>A. Mulleri</i>	<i>A. Thompsonii</i>	2	35
		3	50
<i>A. Mulleri</i>	<i>A. Eclipse</i>	2	60
		3	85
<i>A. Mulleri</i>	<i>A. Mulleri</i>	2	65
		1	60
<i>A. Mulleri</i>	<i>A. megapot. var.</i>	1	105
		2	90
<i>A. Mulleri</i>	<i>A. Gard. Stock</i>	2	50
		4	85
<i>A. megapot. var.</i>	<i>A. Thompsonii</i>	2	50
		2	65
<i>A. megapot. var.</i>	<i>A. Eclipse</i>	1	70
		2	50
<i>A. megapot. var.</i>	<i>A. Mulleri</i>	2	90
		1	80
<i>A. megapot. var.</i>	<i>A. megapot. var.</i>	5	90
		2	110
<i>A. megapot. var.</i>	<i>A. Gard. Stock</i>	2	80
		1	90
<i>A. Gard. Stock</i>	<i>A. Thompsonii</i>	4	60
		2	85
<i>A. Gard. Stock</i>	<i>A. Eclipse</i>	4	90
		2	75
<i>A. Gard. Stock</i>	<i>A. Mulleri</i>	2	85
		1	60
<i>A. Gard. Stock</i>	<i>A. megapot. var.</i>	1	100
		2	130
<i>A. Gard. Stock</i>	<i>A. Gard. Stock</i>	2	35
		4	50

change in character or degree of the variegation is noticeable in either scion or stock. But when an *Abutilon Thompsonii* scion is grafted on a normally variegated *Abutilon* Garden Stock, the variegation of the latter becomes greatly intensified; white and green instead of the usual yellow and green. Results of the above mentioned grafts are shown in plates 3 and 4.

It is evident from the above, that the type of virus present in *Abutilon* Garden Stock differs from that in *Abutilon Thompsonii*, as shown by symptoms produced in the same susceptible stock and that *A. Eclipse*, *A. Mulleri* and *A. megapotaemicum variegatum* possess the same type of virus as *A. Thompsonii*.

DISCUSSION AND SUMMARY

The transmission of the virus. In no instance during these studies on the transmission of the virus among Abutilons did the variegation appear in normally green plants without being grafted. Hence the insects which were present in the greenhouses of the New York Botanical Garden did not transmit the virus to healthy green plants during the period when these experiments were conducted. This does not exclude the possibility of the existence of insect vectors where Abutilons are indigenous.

When variegated scions were grafted on green plants of the various Abutilons tested and vice versa, transmission occurred in all cases. No immune clons were found although differences in susceptibility were noted. Young variegated plants transmitted the variegation more quickly and readily to young green scions than did older ones. When older plants were used symptoms of the virus appeared after a greater lapse of time, creating the impression at first that the green stock or scion, as the case may have been, was immune, but in all instances the variegation did eventually appear.

The delay in the transmission of the virus is evidently due to poor contact of phloem in the area of the union of scion and stock. It was demonstrated by Baur (1906b) that the transmission of this virus and the development of the variegation of Abutilons can be prevented by ringing the bark of susceptible green scions, showing that the causative agent is transported through the phloem tissues. When the ring is allowed to heal, transmission occurs. Hence to obtain ready transmission, it is necessary that phloem tissues of stock and scion establish contact.

The nature of green branches. The entirely green branches which frequently appear on plants otherwise strongly variegated are not immune to the virus. In the case of *Abutilon megapotaemicum variegatum* the extension of the condition frequently results in entirely green plants. Baur

(1906a) claimed that such green branches are immune, but this was not confirmed in the case of green branches appearing on variegated plants of all the clons used in this study. These branches, propagated as green clons, could be reinfected not only by scions of the corresponding variegated clons, but also by scions of any of the other variegated clons.

Thus far, no such green branches have become variegated while attached to the original variegated plant, indicating that the condition which checks the flow of the virus to these branches remains permanent. The variegated parts of plants with green branches were trimmed down in the attempt to induce a flow of virus but in no case did the variegation appear in the green branches. That the virus is not present in green branches of variegated *Abutilons* is shown by the absence of infection when scions from such green branches are grafted on susceptible green *Abutilon* plants. In addition it was demonstrated that the virus does not pass through these branches on variegated plants to infect susceptible green scions. The appearance of green branches is then due to the arrested local distribution of the virus and is in accord with the evidence presented by Baur (1906b) that the virus is not systemic and does not exist in growing points but passes from older leaves to partly matured leaves in succession. Thus the growing points are already free of virus and in the appearance of green branches there is a failure in its flow into the young leaves at the time when they usually receive the virus.

Seed transmission. Definite evidence of the transmission of the virus through seeds was obtained. Of the 3185 seedlings grown which involve all possible combinations of inter-clon pollinations, 461 showed "variegated" characteristics. Of the seedlings tested 4 were capable of transmitting their variegation to *Abutilon Regnellii* and these seedlings were among those obtained from the crossing of *Abutilon* clon Mulleri with *Abutilon* clon Thompsonii, neither of which is self-compatible. Hence, the virus present in *Abutilon* clon Mulleri and *Abutilon* clon Thompsonii is transmitted to a small percentage of their offspring.

In addition to "variegated" characteristics some seedlings obtained from inter-clon crossings showed lacinate and distorted leaves. This characteristic was never transmitted by grafting to the normal green-leaved plants of *Abutilon Regnellii*.

Lindemuth (1878, 1907), Baur (1906a, 1906b), and Hertzsch (1927) reported that the seeds obtained from variegated plants produced none but green seedlings. This result was obtained in my experiments in the case of 969 seedlings grown from seeds of *Abutilon Regnellii* plants made variegated through graft infection. The apparent absence of the virus in the seeds of *Abutilon Regnellii* and the low percentage of infected seed-

lings from other plants indicate that the virus is not usually distributed to the embryo or, if so, that it does not retain its activity there.

Evidence of different strains of viruses. Concerning the existence of various strains or types of the virus in this group of variegated plants, Hertzsch (1927) reported an "A" and "B" type, especially demonstrated by the immunity of *Lavatera arborea* to the "A" type and not to the "B" type. In my studies the two distinct clons (a) *Abutilon* clon Thompsonii and (b) *Abutilon* clon Garden Stock are different in respect to the intensity of the variegation; the *Abutilon Thompsonii* being more intense. When *Abutilon Thompsonii* is used in grafts with *Abutilon* clon Garden Stock the variegation of the latter became intensified. When the two were grafted with plants of seven green stocks two distinct grades of variegation developed, as shown in plates 3 and 4. Thus the presence of two types of virus seems to be demonstrated. These results indicate the presence of two types of virus, but all clons tested were susceptible to both.

This research was carried on at The New York Botanical Garden, from 1928 to 1932 under the direction of Dr. A. B. Stout, and Dr. B. O. Dodge, to whom the writer expresses his sincere appreciation. He is also greatly indebted to Dorothy L. Keur for her able assistance in the preparation of the manuscript.

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Explanation of plates

Plate 1

A green branch of a variegated *Abutilon Thompsonii* was grafted (at A) on a plant of the same variegated clon. The scion grew and the variegation appeared in the young developing leaves of the scion after 172 days (B). This demonstrates that green branches appearing occasionally on variegated *Abutilon Thompsonii* plants are not permanently immune.

Plate 2

This seedling was obtained by crossing *Abutilon Thompsonii* with *Abutilon Mulleri*, both being intensely variegated. A single leaf of this seedling showing its variegation and laciniate characteristics is shown at A. A branch of a green *Abutilon Regnellii* was grafted on this seedling at B. After 37 days the variegation became evident in the newly developing leaves of this scion (C). Normal green leaves present at time of grafting or developing shortly after, are still present on the scion (D). Of all the "variegated" seedlings obtained from the above crossing, only four of those successfully tested were capable of transmitting their variegation to green *Abutilon Regnellii* scions. This demonstrates that the virus causing the variegation of *Abutilon Thompsonii* and *Abutilon Mulleri* is transmissible in a limited way to their seedling offspring.

Plate 3

Above. Effect of the virus of *Abutilon Thompsonii* in leaves of various clons of Abutilon. A. A leaf of *Abutilon Thompsonii* showing character of the variegation. B. A small branch of *Abutilon Regnellii* after graft infection by the *Abutilon Thompsonii* virus. Leaves are small, irregular and largely yellowish-white. C. The effect of the *Abutilon Thompsonii* virus on a leaf of a green *Abutilon megapotamicum* plant, showing large yellowish-white blotches against a green background. Plate 4 shows the characteristics of the variegated *Abutilon megapotamicum variegatum* leaves, very similar to the one pictured here. D. A leaf which developed on a green *Abutilon Eclipse* plant after graft infection by the *Abutilon Thompsonii* virus. The color is yellowish-white, with small green areas. E. A leaf from an originally green *Abutilon Mulleri* plant after graft infection by the *Abutilon Thompsonii* virus, showing yellow and green areas evenly distributed. F. The effect of the *Abutilon Thompsonii* virus on a green plant of *Abutilon* Garden Stock as shown by a typical leaf. G. A leaf of Professor H. Findlay's green Abutilon seedling, after graft infection by the *Abutilon Thompsonii* virus. H. A strongly variegated leaf from an originally green plant of *Abutilon Thompsonii* showing the most pronounced effect of the *Abutilon Thompsonii* virus.

Below. Effect of the virus of *Abutilon* Garden Stock in leaves of various clons of Abutilon. A. Leaf of *Abutilon* Garden Stock showing character of the variegation. B. The effect of the *Abutilon* Garden Stock virus after graft infection on a leaf of *Abutilon Regnellii* showing small yellow flecks and slight distortion. C. The effect on a leaf of an originally green plant of *Abutilon megapotamicum variegatum*. D. The same of *Abutilon Eclipse*. E. *Abutilon Mulleri*. F. A leaf which developed on a green *Abutilon Thompsonii* plant after graft infection by the *Abutilon* Garden Stock virus. G. A strongly variegated leaf from an originally green plant of *Abutilon* Garden Stock showing the most pronounced effect of the *Abutilon* Garden Stock virus. H. The effect on a leaf of Professor H. Findlay's green Abutilon seedling showing very slight variegation.

In comparing these two sets of photographs it is evident that when scions of *Abutilon Thompsonii* and *Abutilon* Garden Stock are grafted on the same series of green Abutilons, the effects produced by the *Abutilon* Garden Stock virus are much less distinct and pronounced than those of the *Abutilon Thompsonii* virus. This indicates the existence of at least two types of virus among the variegated Abutilons used in this work.

Plate 4

A plant of *Abutilon megapotamicum variegatum* on which *Abutilon Regnellii* was grafted (A), showing spread of the virus after 36 days. Green leaves present or just developing at time of grafting are still present (B). A green branch has developed on this plant (C).



KEUR ABUTILON



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The significance of the compiler's data in field work¹

E. D. MERRILL

All who have worked in any of the larger herbaria fully realize the shortcomings of our study collections when information beyond the mere Latin name and localization of a plant is being sought. In most cases the labels bear only the scientific name, the locality, the name of the collector, and the date.

Some years ago, in order to gain a definite idea of the amount of data usually compiled on herbarium sheets, I examined 3,000 specimens at random in three types of herbaria: one local, one economic, and one of exotic material, rich in original collections. In the local herbarium, only about 10% of the labels bore any information regarding the plants themselves; in the economic herbarium less than 3%; and in the exotic herbarium, where facts regarding the plants were especially essential to their understanding, only about 8%.

There is a vast field for improvement in modern herbaria, not so much in material already incorporated, but rather in future accessions. No one interested in economic botany could secure from any existing herbarium much information regarding the economic uses of plants. Neither could anyone interested in local names of plants secure many of these from actual herbarium labels, for the simple reason that collectors seldom record them.

A comparative philologist desiring plant names used by the more primitive peoples would find the average modern herbarium a barren waste. And in what place would he more logically seek such information? The monographer, having available great quantities of material from widespread sources, can frequently gain only the most sketchy ideas as to problems of environment, habitat, degree of abundance of any particular form, altitudinal ranges, soil preferences, and the like, except for the species growing in regions with which he is personally familiar through field work.

An individual working intensively on a definite area automatically assembles in his own mind vast stores of information regarding the plants with which he is familiar. Such information is not ordinarily compiled for incorporation in the herbarium, and his special knowledge usually dies with him. This is not the fault of the individual as much as it is the fault of the herbarium systems, the established methods of which have unfortunately perpetuated themselves.

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That some labels carry information at all is an improvement over the earlier herbarium sheets, for at first the name of the plant was considered the only important thing. The locality and collector were sometimes written on the back. In more modern times, extensive bibliographic references are frequently supplied with *exsiccatae*, but the general information about the plant itself is as conspicuous by its absence as it is in the early basic historical collections.

Consider, for example, the problem of local names. In the eastern United States common names of plants are usually indicated in our manuals. Many of these are perpetuated by the manuals themselves rather than by the people who know the plants. Some never did have the sanction of popular usage; some are merely translations of the specific name or the binomial, and are therefore known only to the author and his readers.

On the other hand, as every country-bred individual who is interested in plant life knows, there are numerous local names of plants, sometimes widely used locally, that never appear in our manuals; they do not appear on our herbarium labels, and under our system or lack of system there is little chance that they ever will be so compiled. Is not a name in actual use for a particular species to be preferred to a coined name? And is it not worth while so to modify our field and herbarium methods that data of this type will actually be incorporated in the herbaria where they will be available for use?

I strongly suspect that the one factor that has inhibited the incorporation of field notes in the herbarium is the notebook system used by many collectors. The data are frequently compiled as notes, but this is as far as the information gets. On his return from a trip, or at the close of a season's field work, the average collector finds that he lacks the time necessary to transfer his notes *in toto* to his herbarium labels, so eventually the fate of his data is their preservation in greatly abbreviated form or their complete loss so far as concerns the individual specimens on which the notes were based. Never having been inoculated with the notebook germ, I have been in a position to consider a substitute system.

In 1899 I found that a field label of sorts was being used in at least one office of the United States Department of Agriculture. The curious thing here was that while these labels carried much detailed information, they were usually not associated with the herbarium sheets and very frequently only the most abbreviated notes were transferred to the herbarium label; the field labels were temporarily preserved in number sequence, but eventually most of them went into the waste-basket.

In some institutions where duplicate copies of field labels were supplied by me supplementing the conventional field label, I have observed

that these were actually discarded by the mounters, or if preserved were not attached to the sheets. Some curators apparently look on mounted herbarium specimens as art objects not to be disfigured by the addition of anything that will not go on a conventional label.

In 1902 I devised a distinctly crude label for use in the Philippines, where the services of individuals not trained in botany were being utilized for botanical exploration. Later in the year, while in Java, I became familiar with the field label developed by Doctor Koorders, and a slightly modified form of this was adapted to our Philippine needs. With the use of field labels a tremendous amount of information was compiled, and these original field notes were in all cases attached to the study-set in the Bureau of Science herbarium. Thus when actual plant work was commenced in preparing my "Enumeration of Philippine Flowering Plants," I actually had before me as I worked over the sheets all the data that were compiled in the field by the numerous collectors, covering habitats, altitudes, relative abundance, soil preferences, colors of flowers and fruits, and other information of this type that the dried specimen itself did not show, as well as local names and economic uses. Thus, of approximately 12,000 native plant names listed in that work, more than nine-tenths of them were taken directly from the herbarium sheets.

One striking thing about the field label is that rarely will a trained botanist adopt it voluntarily, especially if he be accustomed to the notebook system. I must confess that I did not use it consistently until some 8 to 10 years after all the foresters, rangers, and amateur botanists with whom I was in contact in the Philippines were consistently using it. I have been informed by the director of one of our most important institutions that he failed in his efforts to get botanists to use the field label, yet non-botanists who collected for him used it freely.

The field label idea has been widely disseminated during the present century and various forms are now utilized by botanists and collectors in many parts of the world, particularly in China, Japan, Malaysia, the Philippines, and Australia. Comprehensive collections assembled when these simple forms have been consistently used are of much greater value for study and reference purposes than are collections without such data, because the botanist having access to the original study-set has before him instead of merely abbreviated notes or no notes at all, a complete file of all observations actually recorded by the collector in the field. In herbarium practice the field labels are attached to the upper left-hand corner of the herbarium sheets, supplementing the conventional label.

Field labels may be modified in an infinite number of ways and adapted to other uses than simply systematic botany. They may be simple or com-

plex, with few entries for the experienced botanist who knows what should be recorded; or more numerous ones to guide novices as to the types of data desirable for a record. In practice, notes can actually be compiled on field labels with greater rapidity than in a notebook; and where necessary or desirable, through the use of carbon paper, duplicates or triplicates can be prepared.

My special plea is for botanists and collectors to be open-minded enough to give the field label a fair trial. We find in practice that not infrequently these original field notes supply the clues to the actual segregation of species. They furthermore supply the botanist with many details regarding the plants themselves that would otherwise not be available to those who largely depend on standard herbarium specimens as a source for their information. One sometimes feels that the vast majority of botanical collectors, themselves very familiar with the plants they deal with, do not take into consideration the needs of their contemporaries and successors who have occasion to study their material. Their specimens are often merely "specimens," like postage stamps in an album, telling little or nothing about the plant preserved. Our needs will be better served by a more comprehensive idea as to what a "specimen" should include, particularly in regard to the supplying of practical information about each plant, to be kept in the form of notes actually associated with the mounted specimens.

THE NEW YORK BOTANICAL GARDEN

Aims and objectives of plant introduction of the U. S. Department of Agriculture¹

KNOWLES A. RYERSON

The plant explorer who works for the Department of Agriculture must set out on his journeys with a rather different intent from that of the botanist who is interested primarily in adding to the store of knowledge of the world's flora. He must ask himself constantly not only what is this plant, but would it grow back home and if it did, of what use would it be and to whom; not merely is it new to science but is it new to cultivation? In short, he is never a follower of pure science, but is instead a representative in the field of applied science. To this he must add the accomplishments of a horticulturist and an agronomist for he must be a collector of living material and must be able to send his collection home alive, no matter how difficult the journey or how remote the situation.

This attitude brings about not only a different program but a different mass of results which at first may seem of lesser importance when considered critically. It is possible that in all the years of work of the Division, no more than twenty-five new species have been gathered in by explorers, but it is also true that the plants brought in have touched nearly every economic plant industry of the nation's agriculture.

In this governmental activity three general purposes are readily recognized; first, the introduction of entirely new crops; second, the introduction of additional new varieties of those crops which are already established; and third, the introduction of material for use in the improvement of plants cultivated in field, orchard and garden through breeding and selection.

The emphasis placed on each of these purposes naturally has varied with the development of the country. In the early years, as civilization progressed westward across the continent, unlimited land and a wide range of soil and climatic conditions promised an extremely varied agriculture. The introduction of new crop industries was essential.

With the stabilization of agricultural activities, desire for new or better varieties of crops already cultivated, as for example, better wheats, barleys and oats, became an important factor in plant exploration activities, while at the same time the search for entirely new crops continued. Through systematic exploration, activities of the world's important economic crops have been studied and most of them adaptable to our condi-

¹ Presented in a Symposium on Objectives and Methods in Field Work, Systematic Section, Botanical Society of America, at Atlantic City on December 28, 1932.

tions have been introduced either through governmental or private agencies. Today our problem presents another phase. The expansion of the area of staple crops is being checked; actual reduction is being sought. The American farmer is endeavoring to cut down his total acreage and to improve the efficiency of the acres he does cultivate. He must receive higher yields at lower cost of production for those crops which compete in the world markets; or, if possible, grow crops which are non-competitive. The many factors resulting in higher production at lower costs,—increasing yields through improved varieties and strains and reducing the high annual losses from plant diseases, insect pests and extremes of weather,—offer the plant breeder his opportunity.

The annual toll taken of American agriculture by plant diseases alone is rather staggering. Actual estimates in dollars and cents that are made from time to time are open to varied interpretation. If the losses due to the long list of diseases now damaging our field and orchard crops could be eliminated, the cost of production of these crops could be very materially reduced.

While certain groups of plant breeders are at work on the development of disease-resistant crop plants, others are engaged in breeding pest-resistant types, varieties giving higher yields, resistant to drouth, heat and cold, improved canning qualities, and varieties suited to short growing periods in northern states and short rest periods in southern states. These are but a few of the problems which are under way.

This rapid expansion of the field of plant breeding concerns our plant collector since it has brought with it urgent demands for the wild relatives of all the range of our economic crops from whatever portion of the globe they may be found. Plants that might seem poor when judged alone may be of vital importance now that characters, chromosomes and genes are more fully understood.

To get a more accurate picture of the requirements of breeders as they exist throughout the country, a survey was made several years ago of the most important needs of the workers on federal and state projects for foreign plant material and around these needs the exploration and introduction activities are being largely organized, as a glance at the following list of expeditions of the past few years will show:

Brandes expedition in 1928 to New Guinea for wild relatives of sugar cane for breeding mosaic-resistant types.

Beattie expedition in 1927–1930 to the Orient for blight-resistant types of chestnuts, both for new varieties and for breeding stock.

Dorsett-Morse expedition to the Orient from 1929–31 for soybeans and

other legumes; over 4,000 types of soybeans were received for trial and breeding.

Ryerson-Alderman-Leslie expedition in 1929 to northern Manitoba and Saskatchewan for hardy wild types of native fruits.

Westover-Whitehouse expedition in 1929 to Russia, Turkestan and Persia for wilt-resistant types of alfalfa.

Nixon expedition in 1929 to Iraq for rain-resistant dates.

Dickson expedition in 1930 to Russia for rust-resistant wheats and other cereals.

Ryerson-Westover expedition in 1930 to Spain and North Africa for wilt-resistant alfalfas, citrus and deciduous rootstocks.

Russell-Reddick-Erlanson-Souviron expedition in 1930 to Mexico for wild and cultivated tuber-bearing *Solanums* for breeding phytophthora, mosaic and other disease-resistant varieties of potato.

MacMillan-Erlanson expedition in 1931-32 to Peru, Bolivia and Chile for similar *Solanum* investigations.

In some of these expeditions the Department has had the cooperation of the experiment stations of Cornell, California, Wisconsin, Minnesota and the Dominion of Canada.

In addition to expeditions, an extensive range of materials is being introduced through purchase and exchange from all parts of the world.

While special emphasis is being placed on the introduction of material for the plant breeder, as indicated by these recent expeditions, active work is also being pursued in connection with possible new crops, especially those which do not compete with crops now being grown. These include plants producing rotenone, rubber and ephedrine.

The most extensive single introduction problem is that of range improvement. It affects all the western states. The introduction of grass, legume and browse plants to supplement valuable native species is now receiving attention. Closely related to this problem is the introduction of plants for erosion control and for fire-break purposes, if rather inflammable species can be found.

EXPLORATION FOR ORNAMENTAL PLANTS

In the realm of horticulture, the most rapidly expanding section is that of ornamental plants. Our country is just beginning to overtake the pioneer and homestead period in so far as home and town beautification are concerned. As a hobby, gardening is offering keen competition to golf, and women's garden clubs are more in evidence in many sections than are

those exclusively devoted to bridge. As a people, we are beginning to appreciate the fundamental satisfaction of gardening which the English learned long ago. Commercial floriculture and landscape gardening likewise are developing rapidly. Because of these stimuli, the demands from all quarters for new and interesting flowering and ornamental plants are insistent. In no other field of horticulture is there such an opportunity to introduce entirely new and unknown forms to this country or to serve the plant breeder in the creation of strains peculiarly suited to our various soils and climates. Exploration for flowering and ornamental plants, shrubs and trees, in so far as this country is concerned, is hardly begun, though in England for many years privately financed expeditions have been organized exclusively for ornamental plants, as witnessed by the many expeditions to China during the last quarter century from which have been brought back literally hundreds of rhododendrons, primulas, lilies and other bulbous plants, berberis, pyracanthas, cotoneasters, and bamboos new to cultivation. No governmental expedition with such an objective has been possible here as yet.

BOTANICAL AIDS TO EXPLORATION AND INTRODUCTION

A most cursory study of plant exploration activities reveals the inseparable relationship between them and botany in all its branches. This involves not only a knowledge of the foreign field but of our own conditions and it is to the ecologist and plant geographer that we turn first. The taxonomist is then called upon to furnish data on plants existing in the regions to be studied. The physiologist supplies the data that may determine the choice of the particular species that will solve the problem. And the taxonomist must review the work to prove its accuracy.

The building up of an herbarium as such has not been a primary objective of the plant exploration work of the Department, but as a necessary aid for identification and comparison, the collection and preparation of herbarium specimens is an important part of field expeditions. The specimens are filed with the National Herbarium in Washington, D. C. A special seed collection, in charge of a botanist especially skilled in the identification of plants by seed characters, has been built up over a long period of years. Extensive photographic studies are made in the field. At the introduction gardens permanent living plant collections of some of the more important new plants are maintained.

As has been pointed out, the objective of all introduction activities is to establish valuable new plants in American industries as soon and as effectively as possible. To this end the Department has the cooperation of workers of state experiment stations, botanical gardens, arboreta, private

research agencies, of certain of the commercial institutions, and of a limited number of specially qualified individuals. In the past the facilities of fairly large numbers of private individuals have been used for testing, but the results for the most part have been unsatisfactory.

This discussion has dealt primarily with introduction activities in this country. There is another side to it; it is an international cooperative enterprise in which streams of plant materials flow both ways. The Division of Foreign Plant Introduction serves to a great extent as a clearing house for the requests of foreign investigators for plant materials from this country; it assists research workers of federal and state institutions in sending material to foreign countries. It also endeavors to supply from different sources native American material for which foreign countries can not send their own explorers. Through this mutual exchange many valuable introductions have been made.

DIVISION OF FOREIGN PLANT INTRODUCTION,
U. S. DEPARTMENT OF AGRICULTURE

The box huckleberry as an illustration of the need for field work¹

EDGAR T. WHERRY

The plant here discussed is a low evergreen ground-covering shrub with leaves resembling those of the common box, but with the technical characters of the blueberry family (*Vacciniaceae*). Michaux, in his "Flora Boreali-Americana," named it *Vaccinium brachycerum*, and gave the locality as "Virginia circa Winchester," although the specimen in his herbarium is labelled "Warm Springs," perhaps referring to what is now known as Berkeley Springs, in West Virginia. It was again found by Matthias Kinn, about 1800, in the Greenbrier Valley, and by Pursh in 1805 near Sweet Springs.

Upon the death of these early collectors, the localities from which they had obtained the box huckleberry were lost to science, and for many years Asa Gray was unable to obtain material for study in connection with his monograph of the family. In 1845, however, a colony of it was discovered by Spencer F. Baird near New Bloomfield, Perry County, Pennsylvania, enabling Gray to prepare an adequate description of the species and to refer it to the correct genus, its name becoming *Gaylussacia brachycera*. The friendship which sprang up between Gray and Baird ultimately led to the latter's becoming Secretary of the Smithsonian Institution, so this plant may be said to have played an important part in the development of science in America.

In 1918 Dr. Frederick V. Coville recognized that the isolated colony of the plant discovered by Baird consisted of a single individual, which had spread over 8 acres by means of rootstocks. As these grow on the average but 6 inches per year, the colony must have sprung from a seed which had germinated about 1200 years previously, and was thus to be numbered among the oldest of living things. Being nearly self-sterile, the seeds failed to produce young plants with sufficient vitality to reach maturity, and the species appeared to be in real danger of extermination through clearing of the land and vandalism on the part of the public.² If, however, another colony could be found and cross-pollination be carried out, vigorous seedlings might conceivably be obtained, permitting the introduction of the

¹ Presented in a Symposium on Objectives and Methods in Field Work, Systematic Section, Botanical Society of America, at Atlantic City on December 28, 1932. Contribution from the Botanical Laboratory and Morris Arboretum of the University of Pennsylvania.

² This area has subsequently been made a state preserve.

species into horticulture as well as into protected areas, and its consequent perpetuation. At that time, however, not a single other occurrence was known to any botanist consulted by Dr. Coville, nor was there any information in the literature which would lead to the finding of one. Had a list of American relic-endemics been compiled at that time, it would certainly have included the box huckleberry.

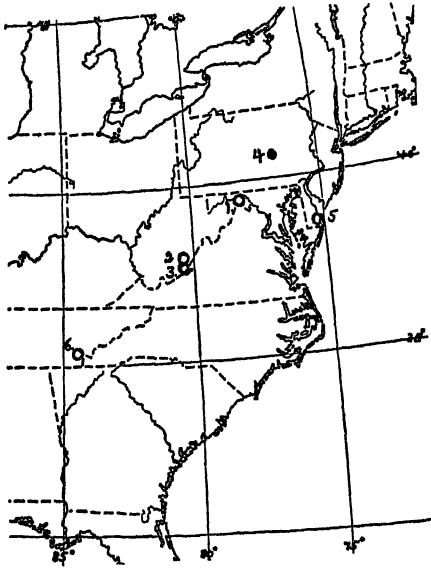


Fig. 1. Supposed distribution of *Gaylussacia brachycera* in 1918. 1. "Warm Springs," Michaux about 1790 = "Near Winchester," 1803; presumably Berkeley Springs, Morgan County, West Virginia. 2. "Krien Preyer," Kinn, 1800; = Greenbrier Valley, east of Lewisburg, Greenbrier County, West Virginia. 3. Sweet Springs, Pursh, 1805; in Monroe County, West Virginia. 4. New Bloomfield, Perry County, Pennsylvania, Baird, 1845. 5. Millsboro, Sussex County, Delaware, Commons, 1876. 6. Parksville, Polk County, Tennessee, Gattinger, 1901. All but No. 4 were lost to science in 1918.

A colony of the species had been found by A. Commons of Wilmington, Delaware, in the southern part of that state about 1875, and although it was reported to have been destroyed, the writer succeeded in rediscovering it in 1919. Cross-pollination between this and a clump from the Pennsylvania colony was carried out, and an article on the plant was published by Dr. Coville.³

Then one after another additional occurrences were brought to notice, —in Pennsylvania a few miles east of Baird's locality, in Maryland less than an hour's ride from Washington, in southwestern Virginia, and so on.

³ Science, 50: 30, 1919.

In 1921 Rev. Fred W. Gray observed it near Dorr, West Virginia, and learning that it was locally known as "juniper-berry" and was used for food by the people of that region, he published in a local newspaper an inquiry as to where the plant so-named could be found. Notwithstanding the fact that the species had not been mentioned in any of the compilations dealing with the flora of the state, he received reports of over 75

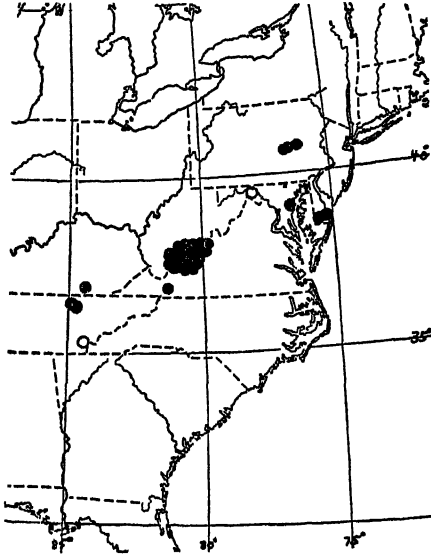


Fig. 2. Known distribution of *Gaylussacia brachycera* in 1932.

Pennsylvania: New Bloomfield; opposite Losh Run Station, also in Perry County, H. A. Ward, 1920; 15 miles northwest of Lebanon, Lebanon County, H. J. Roddy, 1930.

Delaware: Millsboro, rediscovered by the writer, 1919; west of Bethel, also in Sussex County, W. S. Taber, 1932.

Maryland: Pasadena, Anne Arundel County, C. C. Plitt, found about 1910 but not reported until 1920.

West Virginia: Michaux's locality never rediscovered; numerous localities found through efforts of Rev. Fred W. Gray, 1921, in Greenbrier, Mercer, Monroe, Pocahontas, and Summers counties.

Virginia: Several localities in Alleghany, Bath, and Craig counties, F. W. Gray; Fries Junction, Carroll County, W. K. La Bar, found about 1912, but not reported until 1919.

Kentucky: Just west of Cumberland Falls, Pennell and Wherry, 1927; two miles further west, Miss E. L. Braun, 1932, both in McCreary County.

Tennessee: Gattinger's locality never rediscovered; 3 miles east of Allardt, Fentress County, S. H. Essary, 1920; along White Oak River near Rugby, boundary between Fentress and Morgan counties, S. A. Cain, 1930; along Obed River near Rugby, Morgan County, Essary, 1931

stations distributed through five counties, and representing hundreds of acres of ground-cover.⁴ Still later it turned out that in Kentucky the plant is called "ground-huckleberry" and in Tennessee "bear-huckleberry," and no doubt colonies additional to the few now known could be located in those states by similar inquiry under these names. Instead of being a relic-endemic, limited to one or two isolated colonies, as believed for a time, the species actually occurs in at least 7 states, from sea-level on the Coastal Plain to 3000 feet up on the Appalachian Plateau.

The fact that the ranges of native plants are stated with a considerable degree of definiteness in manuals and local floras tends to lead the student and amateur botanist to feel that we are already well informed as to the distribution of species in this country. Circumstances such as those above discussed indicate, however, that such is not the case. When a plant which forms a cover over many acres in at least five counties, and is known to natives as a source of food, fails to be so much as mentioned in a pretentious state flora, the need for more intensive work on plant geography is evident. Teachers will do well to encourage their students who are so fortunate as to live within reach of areas where man has not yet destroyed the native vegetation, to compile local floras with full field notes, including the names used for the plants by laymen. Before theorizing as to the principles of plant distribution, the relation of area to age, etc., let us first find out more as to where our species of native plants really grow.

DEPARTMENT OF BOTANY
UNIVERSITY OF PENNSYLVANIA

⁴ *Torrey* 22: 17. 1922.

How field study can modify older taxonomic concepts¹

F. W. PENNELL

I suppose that many of us have pondered on the psychological behavior of successive generations of taxonomic botanists. We taxonomists are engaged in the fascinating task of trying to discover how the plant-life of the earth is interrelated and how its component divisions are distributed, assembling information that we deem fundamental to the study of plants from any other viewpoint. But the history of our science has not been a steady progress—it has shown rather a rhythmic course, now a period of rapid describing of new species, now a period of critical review in which the tendency has been to merge together what has already been described. In the slang of taxonomy, the rhythm has repeatedly swung from “splitting” to “lumping,” and then from “lumping” to “splitting” again.

First there was necessarily a long period of exploration, during which new species were rapidly described. Perhaps some pre-Linnean author such as Bauhin first arrested this, but much more important was to be the effort of Linnaeus to put in order on a world-wide basis the information that had been acquired by the middle of the eighteenth century. In his “*Genera Plantarum*” Linnaeus based classification primarily upon the structure of the flower, and for this purpose, with his usual methodical thoroughness, he told for each genus whether he had studied it living, had seen it in the herbarium, or had merely adopted it from the account of others.

After Linnaeus came pure exploration again, and at an accelerated pace the multiplication of species. The world was wide, new floras were everywhere being discovered, and it was almost a logical conclusion that what appeared to be peculiar specimens would prove to be new species. From the days of the “*Species Plantarum*” nearly all study was made on dried specimens, for technique had improved while the collector usually had no time to do more than gather material for later inspection. The first flora of North America, the “*Flora Boreali-Americana*” of 1803, was based upon the specimens gathered during the extensive travels of the elder Michaux, but they were studied after his return to Paris and mostly by the French botanist Richard. Pursh, in his “*Flora Americae Septentrionalis*” of 1814, stated whether he had seen the species living, and he may well have made field descriptions. So may Thomas Nuttall for his “*Genera of North American Plants*” of 1818, and perhaps even that ardent field-explorer Rafinesque did so, although his work was too sketchy and impressionistic to gain much benefit therefrom.

¹ Presented in a Symposium on Objectives and Methods in Field Work, Systematic Section, Botanical Society of America, at Atlantic City on December 28, 1932.

But, just as a century before under Linnaeus, so toward the middle of the nineteenth century a vigorous reaction set in; Bentham, the younger Hooker, and Asa Gray (to mention botanists of the English-speaking world) attempted again to revise the immense accumulation of species descriptions, to eliminate duplications, and to get a better view of the taxonomy of the flowering plants. Hooker collected in Great Britain and in India, but his collections were studied as dried specimens at Kew Gardens. Species upon species he decided had been needlessly described, as what had seemed on a single or a few collections to be distinct entities had their supposed characters broken down by the inspection of more material. Finally, he was ready to exclaim: "More specimens always break down characters." In North America Asa Gray, working in the quiet of the Harvard Herbarium, felt equally confident in placing in synonymy hosts of the species of Nuttall, Buckley, and many others, as seeming intermediates appeared in the new collections that were every year flowing to his desk. Before leaving their company, may I say that for wide grasp of the taxonomy of flowering plants we have never had greater masters than Bentham, Hooker, and Gray.

Late in the nineteenth century came a reaction, this time away from the inclusive standards of these workers, and once more toward the rapid multiplication of descriptions of species. Exploration was still continuing, as indeed it had been under Gray, but now in our country instead of the opening west producing all the novelties, they were discerned in abundance in what had been thought to be well-worked fields. Much of the newer work was still wholly of herbarium material, and a multitude of species of Greene, Rydberg, and others, await future confirmation, but we must recall that it was Greene's field observations that led to a new understanding of *Antennaria* and Rydberg's to such evident distinctions as that of *Besseyia* from *Synthyris*. Bicknell carried on the patient observations that showed us so clearly what species of *Sisyrinchium* and *Sanicula* grow in the northeastern states, and Fernald and Wiegand have by field study perfected our knowledge of many groups. The last are still aiding us, but the time draws near for another psychological reaction toward broad inclusive species, unless this newer work (and the conditions of life today) may have placed at our disposal new methods and criteria sufficient for a new kind of systematic study.

Leaving aside from this discussion such special themes as genetical and transplant tests, I want to ask what hope there is for practical taxonomy in field study. The herbarium botanist always wishes that he could actually see in all its details the living plant—it is obvious that any taxonomy would be greatly helped by descriptions of the plants as they grow

in nature. Again, the herbarium botanist, whether conservative or radical by temperament, is ever being puzzled by the odd or strange specimen—it would be of incalculable help if he could see the colonies whence such plants came so as to decide whether they were aberrant specimens or if they indicated some unsuspected distinct racial entity, species or subspecies. Lastly, if we can make the distinction between what are to be recognized as species or subspecies depend upon the presence of intergradation in nature, field study can give us invaluable help in judging such distinctions. I think that no brief is needed to plead the exceeding help of field study to taxonomy, but is such study on a wide scale practicable?

Over some of the world it already is so, and nowhere to such a degree as in temperate North America. The train, and more fully the automobile, have made possible a field taxonomy that may be either floristic or monographic. For the former look at Deam's work in Indiana, where one man, by means of the automobile, has raised his state from comparative neglect to that of the best known botanically in the Union. But I want to urge the prosecution of monographic work by field methods. After all it is easily possible for Mr. Deam, in spite of his thorough field study, to be mistakenly assuming that some Indiana species was the same as another species that was originally described from coastal Virginia, just as I shall soon show you how in all innocence botanists of Florida, of Texas, and of Missouri all had different species that they were all wrong in supposing was the Virginian *Penstemon hirsutus*. It is now possible for us to travel quickly over the entire ranges of many Nearctic genera, studying each species in flower and fruit, seeing its characters with new completeness, and learning its behavior and its distribution.

Species, when understood, tend to occupy definite and natural areas, and field taxonomy will contribute needed precision to our picture of North American biota. But for this purpose I would further urge the amplification of field knowledge by the study of the large amount of material that lies scattered in many herbaria of the United States. Recently, in studying the Scrophulariaceae of eastern temperate North America, I have been amazed to discover how much material is available in state and other local herbaria, and you will find the curators of these usually neglected collections only too glad to have their specimens critically studied. As the localities assembled from all sources are mapped, one may feel confidence in the validity of conclusions nearly in proportion as he finds each species coming to occupy a natural and logical range.

I might have chosen many examples of the modification by recent field study of the older specific concepts of Gray and his contemporaries. Occasionally the older species have failed to be sustained, but usually the

tendency has been to the recognition of finer and more precise entities, and I have selected as an example from the eastern United States what has become of the Grayan concept of *Penstemon pubescens*. From herbarium specimens, and those all too few, Gray quite naturally passed as one species, with range from Maine to Wisconsin, Missouri, Florida, and Texas, plants that as dried specimens seemed greatly alike, similar in capsules, sepals, leaves, and all marked by hairiness on the stem and leaves. But knowledge of the fresh corollas has shown contrasts so strong, that no one seeing them, would wish to hold together this old species; the distinctions of most importance concern corolla-form and color, but, from the clues so offered, one finds that there are also correlated characters of anthers, leaves, and hairiness. The group divides into thirteen taxonomic entities, all of which (because of lack of intergradation) I would prefer to consider species, though granting that a more conservative view could combine them into as few as ten species. Each of these thirteen occupies a definite and natural range.

A specimen, with map of all known counties of occurrence, was shown for each of the 13 species segregated from the former concept of "*Penstemon pubescens*"; these species are soon to be discussed, with reproduction of these maps, in the author's "Scrophulariaceae of Eastern Temperate North America" to appear from the Academy of Natural Sciences of Philadelphia.

Botanists, like other humanity, are subject to psychological laws, yet I think that field methods of systematic study can give criteria and the means of testing them that will greatly modify the next-recurrent swing of thought toward taxonomic conservatism. In the light of the evidence shown you, I challenge Hooker's dictum, for surely, on sufficient study, more specimens may as readily confirm suspected characters as break them down. If Hooker could have traveled over the area between India and Europe, or even into many consecutive valleys of the Himalayas, with time to have studied his species then and there, I wonder if he would not himself have changed what was an herbarium verdict.

We shall always need herbarium workers, for only by their trained skill in comparing plants will it ever be possible to study at all adequately the vast floras of the earth. But let us, with all respect to the masters who have been or who are still with us, recognize that herbarium taxonomy is in its essence tentative—it needs bit by bit to be restudied and reevaluated by knowledge of the living plants in nature.

Field work with the cryptogams, its needs and methods¹

O. E. JENNINGS

The non-vascular cryptogams are much less known to botanists than are pteridophytes and spermatophytes. For the latter, size and apparent abundance have insured early and extensive attention, and their study is considered fundamental in botanical courses. But the systematic botany of the so-called "lower plants" is considered as of a more advanced and specialized character, and for good reasons.

In the first place, there are as yet very few general manuals of the large groups of non-vascular cryptogams which apply to any large area of the country, such as is the case for the pteridophytes and spermatophytes with Gray's "Manual" or Britton & Brown's "Illustrated Flora." Such comprehensive manuals as we have for the lower plants are generally of rather limited systematic scope. Such are the works of Seaver, MacBride and others among the fungi; Wille or Collins among the algae; Grout on mosses. Or else they are decidedly technical, such as Tuckerman for the lichens or Ellis & Everhart for the Pyrenomycetes, or Arthur for the rusts.

Another feature which has contributed to the more specialized character of the systematic botany of the non-vascular cryptogams, is that most of them are small or even microscopic, and special methods and often special apparatus are necessary in field-work, as is particularly the case with certain of the groups of fungi and algae.

Because of the lack of comprehensive manuals for the non-vascular cryptogams and the more advanced and technical training for their collection and study, the work has been largely left to specialists. Few institutions and few localities possess a requisite number of such specialists to represent the various groups. One institution will have a local herbarium rich, for instance, in fungi, or in some group of them, but poor in other groups. The distribution of exsiccatae sets of specimens in lichens, fungi, and bryophytes, has helped, but many know the difficulty in using exsiccatae specimens from distant states. How much more satisfactory would have been an authentic and comprehensive local herbarium with abundance of material!

It is too much to expect, that we may have in the immediate future well illustrated, comprehensive, and thorough manuals for fungi, algae, or bryophytes. There is not yet the abundance of material available on which to base such manuals.

¹ Presented in a Symposium on Objectives and Methods in Field Work, Systematic Section, Botanical Society of America, at Atlantic City on December 28, 1932.

We need (1) a much greater representation of localities in the collections of the lower plants, (2) a much greater number of workers in the various groups, and (3) comprehensive manuals of greater systematic scope and wide geographic range. To attain these three general objectives we should stress emphatically the value and the need of detailed and intensive work on the flora of restricted geographical regions. Even a state is generally too large for the best work.

In 1905 I began collecting, building up a herbarium, and studying the mosses of the western half of Pennsylvania, an area about 150 miles square. Many trips were made, all counties were visited, and special attention was paid to ecological habitats. Eight years later I published an illustrated manual of the mosses of the region, including 248 species. Many species, such as *Stereodon imponens* and *Dicranella heteromalla* were represented by dozens of specimens from various localities and collected during practically all months of the year. Other species were collected but once. Notwithstanding the thoroughness with which this work was done, there have since been recognized more than 20 species in the same area. I had missed 8% of the moss flora of the region. Instead of the 248 species included in my Manual, there are probably at least 300 species in the region. Even yet there are considerable numbers of the species which I can not recognize in the field. They must be taken "on suspicion" and studied later under the compound microscope. This makes it all the more imperative that most careful and concentrated work be done in the field when dealing with such groups of the plants. When one is looking for specimens of forest trees he is likely to overlook some of the smaller mosses or liverworts.

In the field of the bryophytes, we do not at the present time have local floras which represent intensive collecting and study from any considerable part of the total area of the United States. One of the most urgent needs in the systematic botany of our country now is a large number of workers scattered over various sections and working on the local flora of some particular group of the non-vascular cryptogams. This might be stated as the great objective in the systematic botany of these lower plants.

METHODS OF COLLECTION

Others on this program have called attention to the need of more exact data on the specimens. In the non-vascular cryptogams, the ecological habitat is often a great aid in establishing the identity of the specimen. Depending on the method of caring for the specimen in the field, care should be taken to insure completeness and exactness of the field-label.

Bryophytes. Mosses and liverworts are easily cared for in the field. Envelopes make satisfactory receptacles for them, and the data of col-

lection may be written on the envelope. The habitat, whether on soil, logs, living trunks, rocks, etc., and the kind of trunk or log or rock should be stated. A common fault is to gather mixed specimens when, with a little care, the same plant could be obtained in nearly unmixed specimens. The plants should be in fruit, if possible. Herein lies one of the advantages of local work, for nearly every month is the distinctive fruiting period of some one of the bryophytes. One should keep in mind the various kinds of habitats preferred by various kinds of bryophytes; ponds, streams, soil, rocks, living trunks of trees, and even different heights on the tree-trunks. Some species prefer knot holes, and, to secure a good representation of *Fissidens*, for instance, many stones in the brook will have to be turned over and scraped on the under side.

Lichens. Soil lichens, such as *Cladonia*, may be placed in boxes in the field. If moist, they may be placed in envelopes as are bryophytes. It is often of advantage when unpacking dry fruticose lichens to moisten them somewhat to avoid breakage. They relax and become pliable immediately. Foliose lichens, if dry and brittle, should also be moistened before placing in the paper pocket in the field. The crustose lichens will have to be broken or chiseled off with the thin surface layers of rock in which they occur. General collections of the average botanist have a very poor representation of the crustose rock lichens! It took a forenoon with a chisel and hammer to collect crustose lichens on a large granite boulder on the shore of a lake in northern Ontario. There were about 20 species, and I would have missed some had I not examined the rock under a lens. Reading glass, hammer, and chisel are needed for such plants.

Fungi. Parasitic fungi on leaves, stems, fruits, or twigs should be collected, together with the suitable part of the host, and placed usually in paper envelopes, taking care to include in the data the identity of the host. Saprophytic fungi, such as some Polyporaceae, should be accompanied by data as to habitat, such as the kind of wood. It is of advantage in connection with the determination of many fleshy fungi to know the habitat. Mushrooms require careful handling. The late Dr. Kellerman used a basket and brought mushrooms home in handmade paper cones rolled on the spot to suit the size of the specimen. Often by the time he reached home there was a spore-print in the cone to indicate spore color. Mushrooms require carefully recorded data, as many of the characteristics, such as colors, attachment of the gills, nature of the surface, and nature of the longitudinal section may be lost or difficult to determine after the specimens are dried. A good guide as to the data to be recorded for mushrooms is to be found in the publication on Michigan fungi by the late Dr. C. H. Kauffman. Unless they are poisoned, naphthelene or other such materials should

be packed with dried collections of fungi, as insects readily infest them. Small boxes, such as pill boxes, are desirable for small fungi, such as most of the Myxomycetes. Perhaps the best method of preserving them is to glue them to the inside of the cover of the box. This may be done before the specimens are dry.

Algae. Larger algae may be floated on paper and mounted like flowering plants. Smaller types, such as most of the inland green algae, should be preserved in bottles. For desmids and diatoms, microscopic mounts should be prepared. Specimens may be placed in a solution of about 2½% formalin (about 4–5% formaldehyde). Many of my earlier collections dried out and were lost, even when placed in small vaseline-coated glass-stoppered bottles. Small bottles of specimens should be placed in a larger jar filled with formalin solution to prevent loss by drying out. For smaller algae special methods must be used. Some of the material may be examined on the spot, with a field microscope so that scums may be skimmed off the surface of the water directly into a wide-mouthed bottle. Smaller forms may be strained out in a piece of fine-mesh cloth and then rinsed into the bottle. A small cloth may be placed in a funnel through which the water is strained and then the cloth itself be placed in the preserving solution. A pipette is useful for bringing up deposits or scums from the bottom of a pool. The potential flora of a pond may be studied by bringing debris or mud into the laboratory and studying and preserving the forms which develop.

It is obvious that no one should attempt to make a collection of all the major groups of plants from a locality on *one* trip, because of the special methods and equipment and kinds of observations necessary for the various groups. It is better to make several trips with the object of confining one's efforts to one group of plants on any particular trip, or to at least only a part of the whole flora.

In conclusion, it is highly desirable that emphasis be placed on intensive local studies and the building up of good herbaria with the aim to publish accurate and adequate local floras and manuals.

CARNEGIE MUSEUM, PITTSBURG, PENNSYLVANIA

INDEX TO AMERICAN BOTANICAL LITERATURE 1930-1933

The aim of this Index is to include all current botanical literature written by Americans, published in America, or based upon American material; the word America being used in the broadest sense.

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A preliminary report on plants treated with the carcinogenic agents of animals¹

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(WITH PLATE 5)

Numerous reports are now available on the effects of various gases, vapors, and fumes on the structure of plants. Sorauer, (1922) on the basis of observation and experimental studies, lists a group of such substances, by-products of industrial activity, which affect the structure of plants. Sorauer found that hydrochloric acid and chlorine cause the death of leaves of the oat and spruce and that the structure of the leaves killed by these gases is different from that of leaves killed by other means. Discoloration and death of leaves have been reported as the common cause of fumes of hydrofluoric acid, nitric acid, and ammonia. Kny, according to Sorauer, has shown that dilute ammonia solutions injure the assimilatory activity of the plant, causing the leaves of greenhouse plants to blacken. He found that only the tissue immediately adjoining the veins of the leaves remains green.

Tar and asphalt fumes arising from street paving have been reported as harmful to such plants as the rose, strawberry, and chestnut. The common effects of the fumes of these substances are the blackening of the leaves, leaf roll, and finally death. Sorauer points out that only the surfaces of the leaves become altered. Injury of the middle of the leaf causes severe leaf roll. Tar fumes acting on *Ampelopsis quinquefolia* for a few weeks produce curling of the young green leaves, while the inner surface of the leaves becomes wrinkled by the development of the tissue lying between the finer ramifications of the veins. Cork-covered surfaces were noted near the mid-rib. With continued injury, the death of the parts of the leaves ensues and perforations follow. Young branches become corky and existing air roots dry up. Attempt at recovery has been noted, when the fumes are discontinued, by the development of corky structures. Sorauer concluded that injuries due to gaseous bodies may be of a chronic or an acute nature. In the former case the organ affected can remain alive by its successful reaction to the stimulus. In the latter case there is a rapid death of the tissue.

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It is evident that the gaseous substances reported by Sorauer in most instances prove toxic and result in the death of the most sensitive organs of the plant. The cork formation reported in mild cases indicates a type of reaction which under more favorable conditions would probably result in active cell proliferation.

The effects of ethylene and illuminating gas on plant growth have been amply reviewed and are of passing interest in so far as the present report is concerned. More recently Denny (1926)¹⁻² tested 224 different chemicals, and approximately 3000 separate experimental lots were used in selecting suitable concentrations and periods of treatment, to determine their effect on the dormancy of the potato. He found ethylene chlorhydrin and the thiocyanates of sodium and potassium gave the best and most consistent results in reactivating the dormant tubers. According to Denny, McCallum suggested ethyl bromid, ethylene dichlorid, carbon tetrachlorid, ammonia, bromine and gasoline as means of arresting the dormant period of the potato.

Wallace (1926-27-28) exposed twigs to ethylene gas for from 2 to 48 hours under a bell jar and then permitted them to remain under the bell jar filled with air. He showed after a period of time, varying from a few days to several weeks, the formation of intumescences, the destruction of buds, the swelling of the apices and internodes on *Pirus Malus*, *P. ioensis*, and *Ginkgo biloba*. Intumescences which develop on buds and on stems in *Transparent* apple pass through three fundamental changes in the tissue; namely, the solution of the walls, hypertrophy of cells, and proliferation of cells. Another significant observation made by Wallace is that the faintest trace of ethylene, 1 part of ethylene to 100,000,000 parts of air, is sufficient to stimulate the production of intumescences.

In studying the effects of fungicides on leaves of the cauliflower, von Schrenk (1903-1905) found the development of numerous wart-like growths within a week, after spraying with various copper salts, in which ammonia was present. Smith (1917) investigating the mechanism of crown gall formation, after inoculations with *Bacterium tumefaciens*, concluded that the products of the metabolism of the parasite were responsible for the cellular proliferation of the host. These products of metabolism Smith believed to be ammonia, alcohol, and an acid. He believed that tumor growth in plants and presumptively cancer in man and animal are due to the physical changes induced by chemicals, such as aldehyde, acetone, alcohol, acids, alkalies, that are thrown into the cells and diffuse from them as the result of the metabolism of the feeble parasite or symbiont, together with the resultant counter-movements of water and food.

For a long time workers in anilin and paraffin factories and those employed as chimney sweeps have been subject most frequently to cancer of the skin. Coal tar and its products have been suspected of this activity. It was not until the now well-known studies of Yamagiwa and Ichikawa (1916) on the effects of coal tar painting on the rabbit and the studies of Tsutsui (1918) on the mouse, that coal tar was recognized as the irritant responsible for these epithelial proliferations known as tar cancer. The numerous publications relating to tar cancer have been thoroughly classified, and reviewed by Woglom (1926). Seelig and Cooper (1933) have continued this classification and evaluated the significance of the tar studies up to the present year. Here it is only essential to point out that repeated paintings of the skin of the ear of the rabbit or the back of the neck of the mouse with coal tar will produce cancerous growths which prove fatal to the animal. The tar acting as an irritant causes epilation, epithelial destruction, wart formations and finally, in a small percentage of animals, epitheliomas.

Recently Kennaway (1930) has shown that the synthetic hydrocarbon, 1:2 5:6 dibenzanthracene is an active carcinogenic agent producing tumors much more rapidly than coal tar; while Cook, Hewett and Heiger (1932-33) have shown that benzpyrene, another synthetic product, is probably a still more active tumor-producing agent.

The attempt to produce tumors on plants by painting with these carcinogenic agents of animals has not been attempted so far as I have been able to determine from the available literature. Komuro (1930-31) immersed seedlings of *Vicia faba* and *Pisum sativum* in a solution of coal tar for a short period of $7\frac{1}{2}$ to 10 minutes. He observed vacuolization of the cytoplasm and nuclei, and giant cell formation; he also noted abnormalities in chromosome number. As indicated above, plant physiologists have studied the effects of various gases and solutions of them in air and water on the growth of the plant. Smith attempted to find a chemical substitute for the plant-tumor-producing parasite he discovered. The reaction of plants to various chemicals described by recent plant physiologists suggested the need of a more directed effort to test the influence of the known carcinogenic agents on plants.

Again, it seems of importance to produce, if possible, some type of cancer or cell proliferation in plants, which would have some provocative agent in common with a known type of animal cancer. It seems evident from the present literature that plants subjected to substances like ethylene and ammonia, produce only limited growths of abnormal cellular proliferations. It must be borne in mind that while crown gall may become

a malignant plant disease and present some analogies to animal cancer, the writer (Levine, 1931) pointed out that in any group of experimentally-induced crown galls, the large majority of them are only benign wart-like growths.

MATERIAL AND METHODS

To study the reaction of plants to paintings of coal tar, Scharlach red and 1:2 5:6 dibenzanthracene, a large variety of plants were used: *Helianthus annuus*, (sunflower); *Ricinus communis*, (castor bean); *Nicotiana tabacum*, var. *Burley*, (tobacco); *Datura stramonium*, (Jimson weed); *Solanum lycopersicum*, (tomato); *Solanum melongena*, (egg plant); *Rosa* sp. var. *Van Fleet* (rose); *Salix fragilis*, (crack willow); *Bryophyllum calycinum*; *Opuntia Keyensis*; *Carnegiea gigantea*, (tree cactus).

The annuals used in these experiments were comparatively short-lived approximately five to seven months. At about May first, seedlings were set out in the garden at Montefiore Hospital, devoted to these studies. Potted plants of the cactus species and *Bryophyllum* were imbedded in the earth at the same time. Cuttings of the willow had been set out in the previous year.

The carcinogenic agents were suspended in ether, pentane, or olive oil. Suspension varied in concentration from 0.1% to 10%. In a large number of cases, the concentration of the suspensions used was similar to that applied in animal-tar studies. The suspensions were painted on the apical area of the plant or in regions where the growth is usually active. The painting was made with a camel's hair brush on one side of the stem, and the same area was repeatedly painted at stated intervals. In some experiments with *Ricinus* and *Datura*, solutions were injected into the lumen of the stem. Paintings were generally preceded by slight injuries similar to those made when inoculating with *Bacterium tumefaciens*. Plants painted without previous injury were also studied. A large series of plants was painted once, while an equally large series was painted once weekly and still another series was painted three times a week.

Control studies were made on a comparatively large group of plants. Some were inoculated with *B. tumefaciens*, others were injured only in the usual manner. Since the suspension of the carcinogenetic agents was made in ether and pentane, a comparatively large number of plants was injured and painted with ether or pentane. These plants were painted as often as those which were painted with tar or Scharlach red.

A solution of 1 part of strong ammonia in 10 parts of distilled water was also used to contrast the effects of this substance with various concentrations of the carcinogenic agents. Approximately 300 plants with

935 painted or injected stems or petioles were studied. I am reporting at present on the gross effects produced by these carcinogenic agents. Plants with single or two or more paintings were fixed in preparation for a microscopical examination of the tissues. In a subsequent communication, a more detailed statement will be made regarding the microscopic changes observed.

OBSERVATIONS

The following report deals with the effects of painting localized areas, or injecting into various organs of growing young plants the carcinogenic agents, coal tar, Scharlach red, or 1:2 5:6 dibenzanthracene. The procedure used, followed in general the methods employed in producing tar tumors on rabbits and mice, modified by the method generally employed in producing plant tumors by the use of *B. tumefaciens*.

Multiple paintings with coal tar and Scharlach red

Paintings on sunflower, *Bryophyllum*, castor bean, tobacco, Jimson weed, crack willow and cactus were started early in June and continued for a period varying from one to five months. Paintings on the *Bryophyllum* and cactus are still in progress in the greenhouse. The paintings were made in some cases once a week, while in other series of studies three weekly paintings were given. The area chosen was generally the apical or growing portion of the plant. The rapidity of growth of the sunflower and its early maturity offer a splendid opportunity to study this plant in detail in a comparatively short time.

Painting the apical rosette of the sunflower, at the time the plants have attained a height of 3 to 4 feet, with a 10% solution of tar in ether results (fig. 1) in the dwarfing of the shoot. The internodal spaces remain short; the margins of the new leaves are somewhat altered. This has been observed in all the plants studied. Repeated, and in many cases, single paintings of the apical part of the growing shoot, result in the dwarfing and finally in the death of the shoot.

When the painting with 10% tar or 1% Scharlach red in ether has been confined to the regions below the growing point, normal development is interfered with in this area. The growth, instead of being positive heliotropic, becomes lateral (fig. 1). In some cases this lateral growth is of short duration. It seems that the distal cells not affected by the paintings, continue their growth normally and the stem straightens out and becomes negative geotropic again. In many cases the growth of the painted area is lateral while the new growth of the terminal portion of the plant is vertical. The lateral growth of the painted area, it appears, is due to the

more rapid growth of the unpainted surface. There is marked swelling of the painted area (figs. 3, 4, 5) and thickening of the tissues about the injuries made by the needle. The swellings become noticeable in four to five days, for the punctures made are prominent at first, and are soon surrounded by what appears to be young callus tissue. This growth is rapid and the small hole made by the needle is closed, giving the appearance of wart-like structures frequently observed in woody twigs after inoculation with *B. tumefaciens*. In non-injured but painted stems, a smooth thickening is noted (fig. 2) above the area painted, while the painted region itself appears smaller in diameter.

Cross and longitudinal sections of sunflower stems injured and painted once weekly for two months or more show increased thickness of the wood and cortex while the pith is much reduced in size. The part of the plant penetrated by the needle becomes surrounded by a thick layer of woody tissue which is continuous with the thickened wood of the stem.

Coal tar (10%) and Scharlach red (1%) suspended in ether or pentane produced similar results on the plants treated, although the majority of the plants painted with ether and the carcinogenic agent show greater reaction than those painted with pentane in which the animal-cancer-producing substance was suspended.

Painting with ether or pentane alone, preceded by injury, interferes with the growth of the apical portion of the stem but the reactions are comparatively smaller than those produced with tar or Scharlach red. There is a slight callus formation about the injured tissue slightly in excess of the control stem in which the tissue was punctured 5 to 10 times with a sterile needle. Stems of sunflower (fig. 11), Ricinus and Datura injured with a needle previously dipped in a sub-culture of an active strain of *B. tumefaciens* produce the well-known crown galls. Stems, on which no pronounced surface scarring of hyperplastic tissue was formed after painting and injury, were observed and considered as "non-takes." Longitudinal sections of these stems revealed large masses of tissue about the injured internal areas. This is clearly shown in the Ricinus and Datura, which are characterized by large hollow stems. Paintings of tar or Scharlach red, as I shall mention below, induce the formation of overgrowths in the lumina of the internodes in the Ricinus and to a less marked degree in the hollow stems of Datura.

The effects of repeated painting of the sunflower with various dilutions of tar and Scharlach red were studied. These plants were slightly scarified over a given area about 1 cm to 3 cm in length and then painted three times a week beginning August 3rd and continued until September 23rd with 0.5% and 1% solutions of Scharlach red in ether and with 0.5%, 2%

and 4% solutions of tar in ether. Ten plants were painted in each series. Thirty-six shoots of the crack willow were also painted with the same suspensions. The latter paintings were continued to November 4th, when one or two shoots from each series were removed and photographed. Paintings are still in progress on the remaining shoots. The first three or four paintings of the willows result in destruction of the bark and cortex until the wood is exposed. Callus tissue is then formed around the injured painted areas until the wound is entirely closed. This results in a swelling of the stem over the painted area. No globular galls have been formed, although the reaction is far in excess of the controls which at this time, show cork-covered streaks in the stem where the scratches were made in the bark.

The stems of the sunflower in this series were studied after 19 paintings, approximately two months after the experiment was started. The results show that the various concentrations of the carcinogenic agents produced similar results (figs. 6-7-8). There was comparatively no difference in the size of the swellings of the stems over the painted areas. The reaction in the larger plants, however, was comparatively greater than in the small thinner shoots. Longitudinal sections through the painted portion of the stems show considerable hyperplasia of the wood tissue and in some cases small wart-like bodies were observed on the surface of the stem, comparable to poorly developed crown galls.

The pith was broken up by hyperplastic areas which extended from the cortex and wood. It is clear from these studies that the repeated paintings of tar or Scharlach red suspended in ether produced similar effects on plants; these studies show further that various dilutions of these agents are similar in their effects on plants. It is also evident that malignant growths analogous to those produced by tar on mice or rabbits are not produced on the species of plants studied, though the duration of painting was comparatively longer than that necessary to cause cancer on animals. Furthermore, the carcinogenic agents of animals do not call forth the large tumor-like growths induced by inoculations with *Bacterium tumefaciens* in plants grown under similar conditions. Nevertheless these striking reactions which result from paintings with tar and Scharlach red indicate clearly that plant tissues are stimulated to proliferation by these agents.

Weekly paintings of the cactus with 10% solution of coal tar started on June 3rd, have yielded no visible external overgrowths although depression of the painted areas was noted, after one month. The failure of these plants to react promptly is expected in view of the fact that crown galls artificially induced in these plants develop very slowly as shown in the studies on *Carnegiea gigantea* (Levine, 1933) and *Opuntia Keyensis* (in press).

The leaves of fourteen plants of *Bryophyllum*, young and old, were painted either with 10% coal tar in ether or 10% coal tar in pentane. The leaves turned brown, showed leaf roll, and died after a month. In another series of tests with this species the stems of 20 plants were painted with 10% coal tar or 1% Scharlach red in ether. The latter agent produced swellings or tumor-like growths on the stems which are still in the process of being painted. The effects of the coal tar are not as striking as those induced with Scharlach red.

Single paintings with coal tar and Scharlach red

Early in the studies of the effects of tar and Scharlach red on plant tissues, it was noted, as mentioned above, that in some cases, a single painting often caused wilting of the injured and painted shoot, and the complete death of the organ followed. Even where the injury consisted of two or three scratches of the epidermis the painting caused death. It appeared to me that the repeated paintings destroyed many of the newly formed cells, thus preventing the formation of globular galls, and that stouter organs of the plant, such as the basal parts of newly formed shoots or well-formed petioles, would respond more favorably to a single painting. The effects of a single painting preceded by slight injuries were studied. A series of sunflowers, castor beans, tomato plants, Jimson weeds, tobaccos, and egg plants (15 of each species) were selected for this test. Ten per cent coal tar in ether and one per cent Scharlach red were used. The same method was followed as outlined above. As control, a smaller number of plants, in each group, was injured without painting and another small group of plants of the same species was inoculated with *B. tumefaciens*. The control-injured plants produced long scars as the stems elongated. The painted plants showed retardation of the area painted in less than seven days. The stem in the sunflower showed marked swelling and the edges of the points of injury became thicker. One month later all tar or Scharlach red had disappeared as far as could be seen with the naked eye. There was evidence of retardation of growth over the painted area. A slight bend of the stem at the painted area was clearly seen. This, however, had disappeared at the time the plants were photographed two months after painting. The painted surfaces of the stems were covered with a scaly, bark-like structure and small intumescences covered with a thin layer of epidermis were noted (figs. 9-10). Cross and longitudinal sections show excessive thickening of the woody structures with marked growth of wood into the pith about the areas injured. The tomato, tobacco and egg plant responded similarly but to a slighter degree; the greatest reaction occurring in the tomato and least in the egg plant. The *Ricinus* stem

(figs. 14-16) one month after painting is swollen and shorter. Its surface is pigmented and scaly. On making longitudinal section of the stem, the lumen of the internode painted is completely filled with new tissue, arising from the inner lining of the stem (figs. 13-15). The reaction of the *Datura* was like that of the *Ricinus* but less marked.

Single paintings with 1:2 5:6 dibenzanthracene

In mid-August a large number of plants were subjected to a single painting with a suspension of 1:2 5:6 dibenzanthracene rubbed up in olive oil. The suspensions were made approximately as follows: 10%, 2%, 1%, 0.5%, 0.1%. For these studies the sunflower, castor bean, Jimson weed, tobacco, and tomato were used. For the purposes of studying the effects of the agent on woody perennial plants, the crack willow and Rose were also used. As in the previous studies, the growing parts of the stem were used. The paintings were preceded by slight injuries such as pricking or scratching the surface of the organ with a sterile needle; then the area was covered with a suspension of the agent. Care was taken to inflict only very slight injuries. One painting was given all the plants mentioned except the sunflower which was painted twice, twelve days apart. The areas painted were rather small approximately $\frac{1}{2}$ cmsq to 1 cmsq.

The more concentrated suspensions produced necrosis and in some cases, as in the tobacco, caused the leaf, shoot, or petiole to break off. The development of the growing point was entirely suspended and necrosis followed, whereupon side shoots were soon formed from axillary buds. Painting with 1%, 0.5% or 0.1% also caused some necrotic area about which fresh pale green colored tissue was formed about the borders of the injuries, especially in the rose, *Datura*, tomato, and *Ricinus*. The majority of cases of the rose and willow showed cork-covered scars over the injured area with marked swellings of the painted portion of the stem (fig. 12). The sunflower and *Daturas* showed the greatest reaction. Here comparatively large swellings in the nature of thick callus tissue, were formed. Longitudinal sections of the *Datura* and sunflower stems showed the pith invaded by the pale green parenchymatous tissue. Besides these swellings of the stem and the internal formation of callus tissue no distinct globular masses comparable to crown gall were formed. The reactions, however, were in all cases much greater than in the controls.

Single injections with various agents

The hollow structure of the *Ricinus* and *Datura* stems is well adapted to injections with various agents. I have also perforated the stem and heads of the sunflower, the stems of the tobacco, and the green tomato

fruits with a large hypodermic needle, which acted as a delicate gouge, removing a fine cylinder of the tissue and thus leaving a hole or canal into which the agents in question were introduced. For these experiments 0.5%, 2%, 4%, and 10% coal tar suspended in ether were used. One part of ammonia to 10 parts of distilled water and 1% Scharlach red in ether were also used. From 0.5 cc to 1 cc of the substance was introduced by means of a fine hypodermic needle.

One hundred and forty injections were made. Twenty-four hours later it was noted that a number of *Ricinus* plants were severely affected; the petiole and in some instances the upper part of the stem of the plant wilted and died; this was especially noted when ammonia and the 2% ether and tar were used.

A number of tomato fruits large and small injected with a 10% or 4% solution of tar in ether, became soft, affected with mold, necrotized, and fell from the vine. A number reached maturity, colored quickly, and appeared normal except that a depression in the area of injection was present. Scar tissue was formed but no excessive gross proliferations were noted.

The injected *Ricinus* (figs. 17-18-19-20-21, see description of figures for explanation) and *Datura* stems showed extensive proliferation of the lining cells of the lumen; proliferation of the cells about the point of inoculation formed a callus which showed small intumescences. The reaction of the lining cells of the lumen in the *Datura* was less marked than in the *Ricinus*. In several instances the insertion of the needle at the point of bifurcation of the *Datura* stem was sufficient to cause it to split in half. The split stems opened and separated by subsequent growth. The inner surfaces which were subjected to ammonia showed a smooth layer of newly formed tissue. The new tissue is brown and has the appearance of cork although green parenchymatous tissues below the outer layer were found. In a number of cases the splitting of the stem extended only for a short distance along the stem. Here callus-like-growths appeared which tended to close the wound in the stem. This occurred in cases where the lumen was injected with tar and ether solutions.

No difference has been noted in the effects of the different solutions injected. The larger the stem, the greater appears the reaction. The absence of the characteristic nodular growths which result after *B. tumefaciens* inoculation indicate that all cells exposed respond alike; this is unlike the reaction obtained with the parasite. There is unquestionably a reaction to the stimuli but it is less marked than in the crown gall disease. Injected sunflower heads and stems and tobacco stems show apparently little neoplastic reaction. The sunflower heads turned brown and died

while the tobacco survived but formed only slight scar tissue. Abundant material from all plants studied in these experiments has been prepared for microscopic examination.

DISCUSSION

The belief, that the mere presence of the bacterial parasite is insufficient stimulus to give rise to the plant neoplasm, known as crown gall is generally accepted. Smith (1917) believed that the chemical substances, produced by the parasitic organism in its metabolic processes, are more likely the active agents. When he injected the chemicals, ammonia etc., formed by the organism *in vitro* and presumably *in vivo*, into the plant he obtained intumescences, in the lumen of the *Ricinus* stem. Smith contended that the ammonia remains in the hollow of the stem and offers a prolonged stimulus to the plant tissue. The action of the carcinogenic agents should be of longer duration than the ammonia. Yet the overgrowths produced in *Ricinus* treated with tar, Scharlach red and dibenzanthracene, appear to be only slightly greater than the reactions obtained with ammonia injections. Single or repeated surface paintings after slight injury, or single injections into the lumen of such plants as *Ricinus* and *Datura* give similar results in my studies irrespective of the concentration of the agents used.

Smith's hypothesis fails to explain why the cortex and cambium in the *Ricinus*, sunflower etc., painted or injected with the carcinogenic agents of animals, or injected with ammonia, fail to form a distinct globular gall on the surface of the plant similar to that produced by the crown gall organism. The neoplastic tissues resulting from the chemical irritants are developed in the tissues, especially in stems with lumina and, according to Smith, are comparable to those formed by injections of suspensions of *B. tumefaciens* in distilled water. It appears that the presence of the organism in the tissue may have some unknown function in tumor formation other than producing the irritating substances. This further separates the neoplasia of plants from cancer of man and animal. The injury used to introduce the organism of the irritant into the host tends to initiate cell proliferation. The chemicals seem to cause cell proliferation for a limited number of generations. While the causes of cancer are still unknown, cell proliferation in neoplasia of man and animal is apparently without limit.

A careful study of my material shows that the cambium or meristematic tissue of the plant responds to the carcinogenic agents and suggests further that the rate of cell proliferation is equalled by the capacity of these cells to differentiate and age so that the mature structures of the stem are rapidly formed. In the crown gall tissue, it appears, the rate of

differentiation is much slower than cell multiplications. Differentiation is completed with the ageing of the plant tumor, converting the mass of young parenchymatous cells into wood and cork. In cancer, only cell proliferation occurs, with little or no differentiation.

So far, large superficial malignant galls have not been produced in plants by chemical means comparable to those induced by *B. tumefaciens* or other parasitic fungi or nematodes. The malignancy of the neoplastic tissue formed by paintings or injections of tar, Scharlach red, 1:2 5:6 dibenzanthracene or the injections of dilute solutions of ammonia is questioned. Swellings, wart-like growths and large internal intumescences in plants have been produced by the carcinogenic agents of animals. Some very few overgrowths formed in the sunflower reported above are only suggestive of crown gall.

It appears from an analysis of the material that the optimum action of the plant to the animal carcinogenic agents may be obtained with a single painting. This leads to the conclusion reached by Leitch (22), who stated that the bias toward malignancy is probably given the cell during the preneoplastic stage. Findlay (1925) showed that mice painted once with hot tar, produced epitheliomas in 3 out of 75 cases. *Helianthus* and *Ricinus* stems painted once with tar or Scharlach red, produced, two months later, swellings and internal overgrowths that are as well developed as those painted 12 to 20 times during a similar period.

It appears that further study should give rise to new methods of chemical or physical stimulation of the plant which will result in the formation of malignant growths like those produced by *B. tumefaciens*. Such results may throw new light on the question of cell proliferation and may possibly clarify our conception of the cancer problem.

It seems, from these studies, that the reaction of plants to the carcinogenic agent of animals is limited. The protective response of the plant to the irritants employed in these experiments is greater than that reaction induced by mechanical injury, but is not so great as that induced by *B. tumefaciens*.

SUMMARY

A report is made on the gross effects of painting or injecting carcinogenic agents of animals, coal tar, Scharlach red, 1:2 5:6 dibenzanthracene on the following plants: *Helianthus annuus*, (sunflower); *Ricinus communis*, (castor bean); *Datura stramonium*, (Jimson weed); *Nicotiana tabacum*, var. *Burley*, (tobacco); *Solanum melongena*, (egg plant); *S. lycopersicum* (tomato); *Bryophyllum calycinum*; *Rosa* sp. var. *Van Fleet*; *Opuntia Keyensis*; *Carnegiea gigantea*, (tree cactus); *Salix fragilis*, (crack

willow). More than three hundred plants were treated with these agents suspended in ether, pentane or olive oil in different concentrations. Some plants were given a single painting while others were painted once weekly, others thrice weekly, for periods from 2 to 5 months. Plants studied were injured prior to the painting so as to simulate conditions generally employed when inoculations are made with *Bacterium tumefaciens*. A large series of plants were injected with various dilutions of tar or solutions of ammonia in water.

The paintings result in a proliferation of tissues about the injured and painted areas giving rise to swelling of the stem with small irregular masses of new tissue. Longitudinal sections of the painted stems show thickening of the woody elements, new wood tissue formation with abundance of parenchymatous tissue. Painting or injecting of plants with hollow stems show excessive proliferation of the inner lining of the stem filling the entire lumen with new tissue.

There is no difference in effects produced by single or repeated paintings; nor is there any difference in the effects of the different concentration of the carcinogenic agents used.

Control plants grown under similar conditions inoculated with *B. tumefaciens* show the usual external tumor masses typical of the crown gall disease. Plants injured and painted with ether and pentane show structural changes with slight hyperplasias. A more detailed report, fully illustrated, will follow.

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Explanation of plate

Fig. 1. Sunflower, growing point, injury followed by 7 paintings, each painting made at interval of one week, with 10% coal tar suspended in ether.

Fig. 2. Sunflower stem not injured, painted 5 times, each painting made at interval of one week, with 10% coal tar in ether.

Fig. 3. Sunflower stem as in fig. 2, injured before painting.

Fig. 4. Sunflower injured before application of 12 paintings with 1% Scharlach red suspended in ether.

Fig. 5. Sunflower stem as in fig. 4, painted with 10% coal tar suspended in pentane.

Figs. 6, 7, 8. Sunflower stems painted following injury, with $\frac{1}{4}$ % and 1% solution Scharlach red in ether, three paintings per week were made, total paintings 19.

Fig. 9. Sunflower stem painted once with 10% solution of coal tar in ether, painting made after injury on 7/10/33; photographed 8/15/33.

Fig. 10. Same as fig. 9, painted with 1% Scharlach red in ether.

Fig. 11. Sunflower stem inoculated with *Bacterium tumefaciens* on 6/17/33; photographed 8/7/33.

Fig. 12. Rose stem painted after injured with 0.1% of 1:2 5:6 dibenzanthracene in olive oil. Painted on 8/17/33; photographed 11/4/33.

Fig. 13. Longitudinal section of Ricinus stem painted once with 1% Scharlach red in ether, after injury, on 7/10/33; photographed 8/15/33.

Fig. 14. Surface view of Ricinus stem shown in fig. 13.

Fig. 15. Longitudinal section of stem of Ricinus painted with 10% coal tar in ether, after injury. Painted 7/10/33; photographed 8/15/33.

Fig. 16. Surface view of stem shown in fig. 15.

Figs. 17, 18. Longitudinal section of two stems of Ricinus' internodal space injected on 7/31/33 with 0.5% solution of coal tar in ether; photographed 10/7/33.

Fig. 19. Same as figs. 17-18, injected 2% solution of coal tar in ether on 8/1/33.

Fig. 20. As above, injected 4% coal tar in ether on 8/2/33.

Fig. 21. As above, injected with 1 part of ammonia in 10 parts of distilled water on 8/7/33.



LEVINK REACTIONS TO CARCINOGENIC AGENTS

The effects of x-rays on growth and respiration of wheat seedlings

DOROTHY S. FRANCIS

(WITH SEVEN TEXT FIGURES)

INTRODUCTION

The discovery of x-rays by Röntgen in 1895, followed in 1896 by the discovery of radioactivity by Becquerel and of radium by the Curies in 1898, opened a new field for research in physics, chemistry, and biology. Many investigations have been made subsequently to determine the physical characteristics of the radiations and to improve the methods of obtaining them. Researches have been conducted also to ascertain the effects produced by these radiations on matter—both non-living and living.

Radiations from radioactive substances are known to be of three main types: (1) alpha rays, which consist of helium nuclei (positively charged), ejected at high speed; (2) beta rays, consisting of electrons (negatively charged), travelling at very high speed; (3) gamma rays, which, for the present purpose, may be considered to be electromagnetic in nature. X-rays also are of this latter type of radiation, and differ from gamma rays only in wave length. All these radiations have the common property of ionizing matter and may be referred to, therefore, as ionizing radiations.

The action of such radiation on living matter may be considered to take place in three steps, of which the first is a physical effect, known as ionization. This ionization is the result of the interaction between radiant energy and matter whereby certain atoms lose temporarily some negative electricity in the form of electrons and become positive ions. The electrons thus liberated attach themselves to atoms or groups of atoms which thereby become negative ions. It should be noted that only a very small percentage of the total number of atoms in the path of the rays is ionized at any one time. Electrons may be liberated by radiation from any kind of atom; therefore, any substance may be ionized—whether it be an electrolyte or a dielectric; organic or inorganic; solid, liquid, or gaseous.

The presence of these electrically charged ions in a compound facilitates a regrouping of the atoms into new compounds. Accordingly, a secondary effect of radiation on matter is the production of chemical changes. Such changes, however, are not always readily perceptible, since only a small fraction of the total number of atoms is ionized, and any changes which take place under these conditions may be reversible.

Changes in the chemical composition and equilibria of an organism may initiate modifications in the biological processes which occur normally.

Accordingly, the production of biological changes in living matter by radiation may be considered to be a tertiary effect.

The primary effect of radiation—ionization—takes place during the time that a substance is exposed to the radiation. The secondary effect—chemical effect—being subsequent to ionization, requires a certain time for its manifestation, which may be short or long, depending on various conditions. In a complex system the first chemical change probably occurs almost simultaneously with ionization. This then may set in motion a series of chemical changes which are independent of further irradiation. The observable biological change is the end result of these chemical changes. Time is required for the completion of this process. Therefore, there should be an interval (latent period) between the exposure to radiation and the manifestation of a biological change. Furthermore, this interval should depend on the nature of the biological change under consideration, since various biological responses require different lengths of time before they become evident. Thus, in studying eye defects produced by irradiating eggs of *Drosophila* (fruit fly), one must wait until the time when eyes develop in the organism before observing the abnormalities.

From these considerations it follows that radiation may produce many biological modifications in a given test object, and that these become evident at different times after irradiation. Whether these changes are detectable or not depends on several factors, but principally on the dose of radiation administered, the radiosensitivity of the material, and the equipment available for the observation of such changes.

Numerous investigations have been reported concerning the biological action of x-rays and other ionizing radiations. Many of these are qualitative studies which describe certain effects resulting from the administration of radiations, without quantitative definition of the doses employed or the effects produced.

Cytological studies have been carried out by a number of investigators, employing either plant or animal material, or both. The results obtained by a few of these workers will serve to illustrate the general types of changes observed in irradiated material.

Among those who have studied the histological modifications produced by x-rays in plant material may be mentioned Johnson (1926), Komuro (1928, 1930a, 1930b), and Bersa (1927a). Some of the changes described included enlargements and vacuolations of the cells in the meristematic region of the root tips; an increase in the amount of xylem at the expense of the pith; and the presence of a greater amount of suberin than usual in the hypocotyl regions of mature plants. Another alteration mentioned was the formation of enlargements in the root tips of x-rayed seedlings;

these enlargements were referred to as "roentgen tumors." In these tumors were giant cells which pushed away the surrounding cells into other rows, thus producing a disturbance in the alignment of the cells. In addition, there were many cells which were multinucleated or degenerated; some of the nuclei contained double chromosome numbers, abnormal nuclear figures, attenuated nuclear membranes, and vacuolized chromatin masses. Another effect of x-rays was a temporary depression in the frequency of nuclear divisions, immediately following irradiation. This depression seemed to be due to a delay in the inception of mitosis, and not to any change in the time interval necessary to complete any one stage in mitosis. This latter type of disturbance was corroborated by observations of Strangeways and Hopwood (1926), experimenting with chick embryos *in vitro*.

Other qualitative investigations have been carried out to study genetic changes which appear in irradiated material. As representative experiments, those performed by Efroimson (1931), Goodspeed (1929), Hanson and Winkleman (1929), Patterson (1929), Painter and Muller (1929), Mavor (1929), and Stadler (1930) may be cited. Experiments of this kind have been performed on a variety of test objects, including *Drosophila melanogaster* (fruit fly), *Nicotiana tabacum* (tobacco), *Hordeum vulgare* (barley), *Zea Mays* (corn), and other materials. The appearance of mutations, the production of breaks in chromosomes, the non-disjunction of the sex-chromosomes, and the translocation of chromosomes are among the effects reported.

Qualitative experiments have been performed also to determine whether radiation alters the viscosity of protoplasm or of protein solutions. The results of these experiments are conflicting. Fairbrother (1928) reported that irradiation with x-rays brought about a marked decrease in the viscosity of egg albumen. On the other hand, Wels (1924) stated that the application of x-rays effected an increase in the viscosity of serum and globulin solutions *in vitro*. Weber (1923) could detect no change in the viscosity of the protoplasm of *Spirogyra* or *Phaseolus* (bean) after exposure to x-rays. Nadson (1925) pointed out the fact that the viscosity of the protoplasm of *Saccharomyces cerevisiae* (yeast) may decrease at first and later increase, after irradiation with beta and gamma rays of radium.

Similar discrepancies appear in studies which have been made of the effects of radiations on the hydrogen-ion concentration of body fluids. When irradiation with x-rays was severe enough to injure the cells, the reaction of blood became acid, according to Cluzet and Kofman (1925). Pannewitz (1927) reported an acid reaction immediately after irradiation of blood serum, albumen, and globulin solutions *in vitro* with x-rays; but

he stated that this acidosis might be followed by an alkalosis lasting for several days. Fernau (1925) and Kolta and Förster (1926) described an alkaline reaction in blood irradiated with x-rays. Woodard and Downes (1931), however, found no consistent change in the hydrogen-ion concentration of blood of irradiated patients, nor in the blood of rabbits after the administration of lethal doses of 200 K.V. roentgen rays.

The very recent development of quantitative methods for the study of the biological action of x-rays and other ionizing radiations has depended mainly on two factors: (1) the devising of suitable methods and instruments for accurate mensuration of the radiation employed; (2) the improvement of technique and apparatus for the measurement of biological modifications produced by radiations. Considerable emphasis should be placed upon quantitative studies in this field, for it seems certain that information regarding the *modus operandi* of the biological action of radiations may be obtained only by correlating a large number of biological changes with the dosages effecting them.

Radiation may be measured by any given effect which it is capable of producing in matter. Effects which may be utilized include the blackening of a photographic plate, the production of fluorescence in certain bodies, the change in a chemical compound, or the modification of a biological process. Ionization, however, is usually employed as the basis for the most convenient and accurate methods of measuring radiation. It is desirable, of course, to express the measurements in terms of a standard unit. Such a unit was established for x-rays in 1928, and is defined as follows: "The International Unit of x-radiation is the quantity of x-radiation which, when the secondary electrons are fully utilized and the wall effect of the chamber is avoided, produces in one cubic centimeter of atmospheric air, at 0°C. and 76 cm. mercury pressure, such a degree of conductivity that one electrostatic unit of charge is measured at saturation current." This unit is called the roentgen (r).

Recently, a number of papers have been published which give quantitative data on the biological effects of radiations. Some of the experiments reported have been designed to study the effects of varying the conditions of radiation used on the same kind of test object. Other tests have been arranged to study the effects of a constant type of radiation on varying test objects or different physiological responses. For investigation of certain problems of the first category, tests have been carried out to determine the characteristics of different qualities of radiation by comparing the effects which they produce on living matter.

As a criterion of the effect of different qualities of radiation, Packard (1927, 1928) studied the mortality produced in the eggs of *Drosophila*

melanogaster (fruit fly). He found that the mortality curve for these eggs showed the same characteristics, regardless of the quality of radiation. In 1932 he investigated the biological effectiveness of x-ray beams of wave lengths of 0.05, 0.08, and 1.70 A.U., obtained from tubes maintained at 550, 300, and 12 kilovolts, respectively. Using *Drosophila* (fruit fly) eggs and fragments of mouse tumors as test objects, he reported that equal doses of these three qualities of radiation, as measured by air ionization chambers, produced equal effects. He pointed out that this confirmed earlier experiments which he had performed with wave lengths of 0.20, 0.30, 0.50, and 0.70 A.U.

Holthusen and Zweifel (1932) performed a series of experiments on the eggs of *Ascaris megalocephala* in an endeavor to discover if there were any difference in the effects of radiations of different wave lengths on these organisms. They could detect no difference in the degree of injury produced by x-rays, gamma rays, and corpuscular rays.

Henshaw, Henshaw, and Francis (1933) investigated the effectiveness of 165-kilovolt x-rays, 700-kilovolt x-rays, and gamma rays of radium in causing growth reduction in *Triticum vulgare* (wheat) seedlings, and on the mortality of *Drosophila* (fruit fly) eggs. They reported a slight difference in the relative effectiveness of these qualities of radiation, which, however, was not definitely greater than the possible experimental error. Therefore, they stated that these results, taken alone, do not prove that there is a differential effect depending on the quality of radiation.

Lachmann and Stubbe (1932) investigated the effects of hard and soft x-rays on the growth of *Vicia faba* (Windsor bean). They found that for the lower doses (50 roentgens) the soft rays were more effective than the hard rays; for somewhat higher doses (100 or more roentgens), however, the hard x-rays were far more effective than the soft rays. At 500 roentgens the degree of injury produced by the two qualities of radiations began to be somewhat comparable, and it seemed likely that the degree of injury produced by very heavy doses of the two qualities of radiations would be about the same.

Several investigators have attempted to determine whether the effect of a certain dose of radiation on the organism is the same when the radiation is administered over a long period of time as when it is administered over a short period. In other words, do effects of radiations follow the Bunsen-Roscoe law, which states that, for a given degree of response, *intensity* \times *time* = *a constant*? Packard (1926) performed a series of tests, involving the use of 50,000 eggs of *Drosophila melanogaster* (fruit fly), which showed that the Bunsen-Roscoe law was applicable within the limits of the experimental conditions employed.

Roesler and Henshaw (1932), however, found that a less marked effect was produced on *Drosophila* (fruit fly) eggs when the intensity was low and the time of exposure was long than when the same dose was administered in a short time at a high intensity. Ancel (1927), in experiments on lentil seedlings, observed a marked decrease in the effect of x-rays when, for a given dose, the duration of the exposure was increased and the intensity of the radiation was decreased.

A number of radiological studies have dealt with the influence of ionizing radiations on various biological processes. Several investigators have studied the effects of radiation on the growth of seedlings.

Bersa (1926) conducted experiments to determine the effects of x-rays on the growth of seedlings of *Vicia faba* (Windsor bean), *Sinapis alba* (mustard), and *Zea Mays* (corn). In a very few cases he obtained increased growth of the shoots following relatively weak doses of x-rays. But he could detect no corresponding increase in the growth of the root system. In general, he noted that doses as weak as 10 Holzknacht's units caused a marked decrease in the growth of the roots. With doses ranging from 10 to 40 Holzknacht's units, this depression was only transitory. After small doses inhibition of growth was of shorter duration than after large doses.

Reinhard and Tucker (1928a, 1928b) irradiated seedlings of *Vicia faba* (Windsor bean), *Pisum sativum* (pea), and other species of plants. They concluded that the sensitivity of seedlings to x-radiation depends on the species irradiated, the age of the plant when irradiated, the time of day when radiation is administered, and the portion of the plant exposed.

Injurious and retarding effects of x-radiation have been reported by Heeren (1932), who was able to measure very minute changes in the growth of *Vicia faba* (Windsor bean) by means of an interferometer. He pointed out that there was a distinct retardation in the growth of seedlings which were exposed to x-rays of an intensity of 15 roentgens per minute. From the beginning of irradiation he observed a retardation, which persisted throughout the entire duration of the irradiation. As soon as irradiation ceased, there was an increase in the rate of growth of the roots.

The development of apparatus for the accurate measurement of respiration has made possible a new method of investigating the effects of radiations on living materials. This affords an index of the influence of radiations on one of the most fundamental of physiological processes. Several studies recently have dealt with the effects of radiation on respiration. As examples of this type of experiment, those performed by Bersa (1927b) and Adler (1929 and 1930) may be mentioned. They employed different

test objects—*Vicia faba* (Windsor bean) and rat testicles—and used different methods of measurement of respiratory activity; but both reported decreased respiration after heavy irradiation of the material. After weak doses there appeared to be a transitory acceleration in some of the plant material.

Cattell (1931), working in the Biophysical Laboratory of the Memorial Hospital, developed an excellent technique for using the linear growth of the different organs of seedlings of *Triticum vulgare* (wheat) in the study of certain radiation problems. He found that this material possesses several advantages: The radiation effects on the growth of four different organs of the seedlings may be measured within a short time after the administration of radiation. The results may be duplicated from time to time with reasonable precision. Failla and Henshaw (1931) and Henshaw, Henshaw, and Francis (1933), working in the same laboratory, used this organism extensively in studying the influence which the wave length of radiation exerts on the quantitative relation between dose and effect.

Although retardation of the linear growth of the four organs of the seedlings has been found to possess considerable practical value, it must be recognized that this is only one manifestation of the effects of radiation. It is of interest, therefore, to search for other manifestations with the two-fold purpose of acquiring more information about the mode of action of radiation and possibly of discovering some radiation effect which is of even greater biological significance than growth retardation.

The work which is reported in this paper was performed in the Biophysical Laboratory of the Memorial Hospital during 1931 and 1932. The author wishes to express her appreciation to Prof. S. F. Trelease, of the Department of Botany of Columbia University, for many helpful suggestions and criticisms. It is with pleasure, also, that she acknowledges her indebtedness to Drs. G. Failla and P. S. Henshaw, of the Memorial Hospital, for their interest and assistance on numerous occasions.

MATERIALS AND METHODS

The experiments reported in the present study were arranged so that four physiological responses to x-rays might be investigated in the same material—seedlings of *Triticum vulgare* (wheat). Each test covered a period of about seventy-six hours and included irradiation of the experimental seedlings, followed by periodic measurements of growth in length of the four different organs (coleoptile, leaf, primary root, lateral roots), determinations of fresh and dry weights of the growing portions, and measurements of carbon dioxide production. The process of germination was started by soaking the seeds in water. After an interval of twenty-four

hours the experimental seedlings were irradiated. Determinations of growth and respiration were made thirty hours, fifty-two hours, and seventy-six hours after the dry seeds had been put to soak.

The technique followed for germination and irradiation of the seedlings was the same for all tests. Seeds of "Kota" wheat were soaked in tap water for three hours, and then planted in glass chambers containing two thicknesses of filter paper moistened with 30 cc. of water. These chambers were placed in a dark incubator at a temperature of 26°C. for twenty-four hours. At the end of this time the seeds had germinated, the coleoptiles being about 2.0 mm. in length and the primary roots about 3.0 mm. At this stage, seedlings were selected for irradiation. Groups of about five hundred seedlings were placed in small cardboard trays which had been dipped in paraffin. Three trays at a time were covered by a filter of 2.45 mm. aluminium and 0.1 mm. copper. They were supported on a celluloid tray at a distance of 35 cm. from the target of the x-ray tube. The latter was a standard, water-cooled, Coolidge tube, and was maintained at a potential of 200 kilovolts and a current of 32 milliamperes. At this setting the output of the machine was 226 roentgens per minute. The trays of experimental seedlings were exposed to x-rays for intervals of $2\frac{1}{2}$, 5, $7\frac{1}{2}$, 15, 30, and 60 minutes, respectively. One tray was not irradiated, but was retained as a control.

Following irradiation, the seedlings were removed from the trays. Those to be used for the measurements of growth and weight were planted in Petri dishes containing two sheets of filter paper and 10 cc. of water. For studying respiration, groups of one hundred seedlings were placed on large glass slides covered with moist strips of filter paper. The slides were put in cylindrical chambers (35×5 cm.) that were kept in the incubator, and connected to the titration flasks of the respiration apparatus.

The study of the effects produced by the x-rays on the seedlings was begun approximately thirty hours after the seeds were soaked—that is, about six hours after irradiation. The linear growth was determined in millimeters for each of fifty seedlings of the controls and of the irradiated wheat. The growing portions of one hundred representative seedlings of each group were separated from the hard storage regions of the seed, and after removal of the excess water by blotting, they were placed in a small beaker and weighed, in order to determine their fresh weight. The same portions were then dried in an incubator at 60°C. for a period of about fourteen days, and in desiccators for nine days. Then the dry weights were obtained. As previously mentioned, determinations of growth and weight were made at intervals of thirty hours, fifty-two hours, and seventy-six hours after the seeds were soaked.

Coincidentally with the measurements described above, respiration was studied in similar groups of seedlings. The carbon dioxide production of the seedlings was used as a measure of their respiratory activity. The apparatus employed was essentially the same as that described by Mack (1930), which, in turn, was a modification of an apparatus described by Carrick (1928). The diagram in figure 1 shows the essential features of the

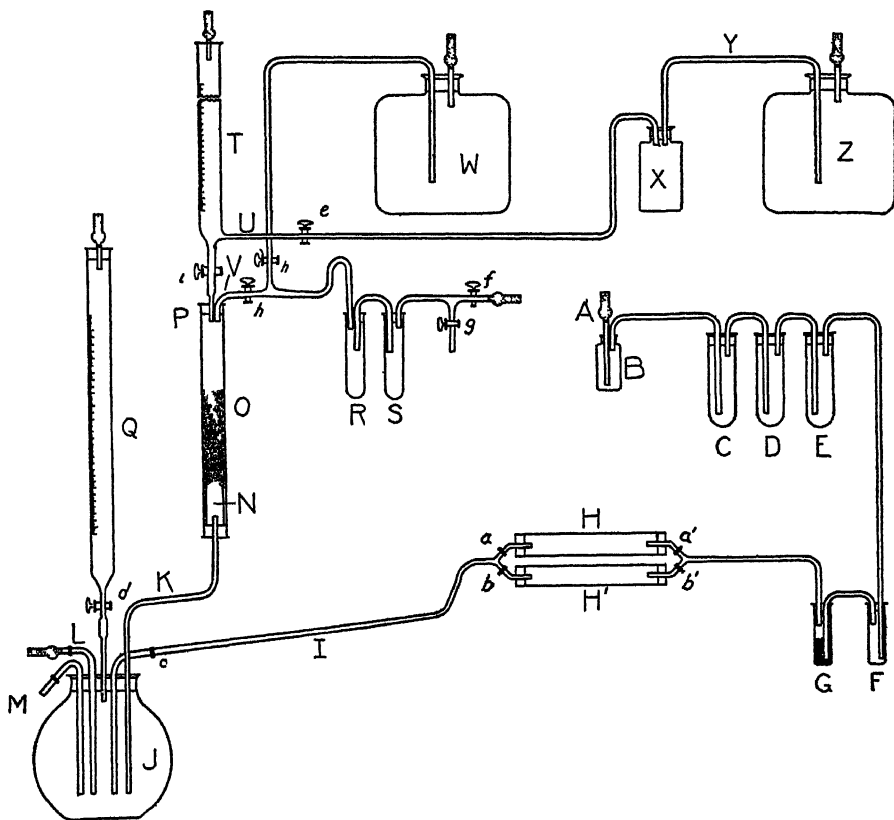


Fig. 1. Apparatus used to determine carbon dioxide production.
Explanation in text.

respiration apparatus employed in these experiments. The apparatus consists of the following main parts: the purification system (A to G, inclusive); the respiration chambers (H and H') containing the seedlings; the titration flask (J); the absorption tower (O); the outlet tell-tales (R and S); the standard acid burette (Q); the automatic burette (T) containing standard alkali solution; the 19-liter reservoir (W) of distilled water and phenolphthalein.

The purification system is designed to remove dust particles and carbon dioxide from atmospheric air, before the latter passes through the respiration chambers, and to maintain a constant humidity for the seedlings. Air from out-of-doors is passed through soda-lime (A); then through a 10 per cent solution of sulphuric acid (B); next, through a 10 per cent solution of sodium hydroxide (C, D, E); and finally, through distilled water in two bottles (F, G), one of which contains a number of glass beads.

The respiration chambers are glass cylinders (35×5 cm.) that are closed at each end by rubber stoppers, through which short glass tubes pass. As shown in figure 1, the arrangement is such that the pair of chambers has one inlet in common, from the purification system, and one outlet in common, to the absorption and titration apparatus. Four respiration chambers were used in the course of an experiment, thus necessitating the use of two absorption and titration devices.

The titration flask (J) is a 500-cc. culture flask, fitted with a five-hole rubber stopper. Through this stopper pass the gas inlet tube (I), a vent (L) guarded by a soda-lime tube, a siphon-outlet tube (M), a glass delivery tube from the standard acid burette (Q), and the tube (K) connecting the titration flask to the absorption tower.

The absorption tower (O) is composed of a heavy-walled glass tube (49 cm. long and 1.8 cm. in inside diameter), within which is a column of glass beads (3 to 5 mm. in diameter) about 20 cm. high resting on an inverted glass vial (N), 10 cm. high, with holes blown in its base. The tower is closed at its upper end by a two-hole rubber stopper, through which pass the end of a Y-tube and the glass delivery tube (P) from the automatic burette (T) containing standard alkali solution. One arm of the Y-tube serves as a delivery tube (V) for distilled water containing the indicator, phenolphthalein, when the stopcocks *h* and *h'* are open. The other arm is bent into a U and forms the outlet into the first tell-tale tube, when the stopcock *h* is open. The first outlet tell-tale tube is connected to a second by means of a U-tube.

The outlet tell-tales (R, S) are test tubes (2×18 cm.) provided with two-hole rubber stoppers and inlet and outlet tubes. They are arranged so that any gas that is not taken up in the absorption tower bubbles through a solution of barium hydroxide before escaping from the apparatus. The second outlet tell-tale is provided with a Y-tube, one arm of which serves as an additional vent, guarded by a soda-lime tube; the other arm is connected by a metal tube to a suction pump which draws the gas through the apparatus. If no white precipitate appeared in the tell-tales, it was certain that no carbon dioxide had escaped from the absorption tower.

The standard acid burette (Q), of 100 cc. capacity, is used only in per-

forming titrations. During the course of an experiment the burette is disconnected from the rest of the apparatus by closing the stop-cock (d). The burette is filled with standard hydrochloric acid solution¹ from a 19-liter stock bottle, at intervals during a day's tests.

The automatic burette (T) is likewise of 100 cc. capacity. The delivery tube passes into the absorption tower at P. The lateral, U-shaped branch of the burette is connected to a long tube (U), which conveys standard barium hydroxide solution¹ from a choke-bottle (X) when the stop-cock (e) is opened; the choke-bottle is filled to a constant level by a siphon connection (Y) from a 19-liter stock bottle (Z), which is guarded by a soda-lime tube.

Before carrying out a series of tests, it was necessary to clear the apparatus of any atmospheric carbon dioxide. This was accomplished as follows: As soon as the seedlings had been placed in the respiration chambers, chamber H was clamped off by Hoffman clamps at a and a'; the stop-cock h was opened; and by opening the stop-cock g and closing f, carbon dioxide-free air was aspirated through chamber H' (which was not clamped). This stream of air was regulated so that approximately 220 bubbles per minute passed through the absorption tower. As carbon dioxide-free air passed over the seedlings, it collected any carbon dioxide present in the chamber and carried it into the absorption tower (O), where it was precipitated as barium carbonate. Thus, in the course of a half-hour, chamber H' was freed of any carbon dioxide which might have entered it when the seedlings were put into it. When chamber H' was clear, it was closed at b and b' with Hoffman clamps, while chamber H was freed of carbon dioxide. During this time carbon dioxide accumulated from the seedlings in chamber H' and thus initiated the period of gas collection for chamber H'. An hour after chamber H' was cleared thoroughly and closed, a Hoffman clamp was closed at c on the gas inlet pipe (I). Chamber H' was unclamped and chamber H was clamped. Aspiration was stopped by closing the stop-cock at g. All barium hydroxide solution remaining in the absorption tower was lowered into the titration flask by opening the vent stop-cock (f). Distilled water containing phenolphthalein was washed down into the absorption tower by opening the stop-cocks h and h'. Then the solution was neutralized in the titration flask (J). In order to insure that there was no barium hydroxide solution remaining in the absorption tower, the neutralized mixture was transferred several times from the titration flask to the absorption tower. As soon as the indicator ceased to change to a red

¹ The solutions used in this work were standardized by Dr. H. Q. Woodard, of the Chemical Laboratory of the Memorial Hospital, to whom the author wishes to express her appreciation.

color, it was evident that no barium hydroxide remained in the tower. When this procedure was ended, the neutralized solution was siphoned out of the titration flask through the siphon outlet (M). Distilled water containing phenolphthalein was then discharged again into the absorption tower and titration flask. If no color change took place, it was obvious that no barium hydroxide remained either in the absorption tower or in the titration flask.

When all barium hydroxide solution had been neutralized and the apparatus had been washed and emptied, a known quantity of 0.075 N (and later, 0.127 N) barium hydroxide solution was run into the titration flask. Aspiration was recommenced by closing the stop-cock at f and opening that at g. The Hoffman clamp at c was opened, and aspiration drew the barium hydroxide solution up from the titration flask into the absorption tower, and drew the carbon dioxide from the respiration chamber through the absorption apparatus. At the end of an hour of aspirating through this chamber (H'), there had been absorbed not only the carbon dioxide produced by the seedlings while the air stream was passing through the chamber, but also all gas which had been accumulated during the hour when no air stream was passing through it (i.e., while it was clamped, during the process of clearing chamber H). In this way the total accumulation of the carbon dioxide gas produced during the interval of two hours was collected from the respiration chambers in alternate hours. At the close of each collection period the clamp at c was closed, and aspiration was stopped. The clamps were removed from the closed chamber to the open one. The vent at f was opened, and the barium hydroxide remaining in the titration flask was titrated with 0.056 N (and later, 0.071 N) hydrochloric acid solution, using phenolphthalein as an indicator. The difference between the amount of acid which would have been necessary to neutralize the original quantity of barium hydroxide, and the amount which actually was required to neutralize the remaining quantity of barium hydroxide solution, was equivalent to the amount of barium hydroxide which had been used to form barium carbonate. From this, it was possible to calculate the number of milligrams of carbon dioxide which had been produced by the seedlings during the collection period.

Determinations of the carbon dioxide production in irradiated and non-irradiated seedlings were performed at average intervals of thirty, fifty-two, and seventy-six hours after soaking the seeds. The results obtained are represented graphically in figure 4. They represent the averages of experiments involving at least three hundred seedlings—in many cases, a considerably greater number.

In order to insure reliable results, numerous tests were made of the ap-

paratus. Frequent titrations of the standard solutions were performed to check any possible changes in their concentrations. The apparatus was checked for leaks by making blank tests, with no seedlings present. Finally, the absorbing efficiency of the apparatus was tested by liberating known quantities of carbon dioxide into the titration flask and absorbing tower. The quantities recovered from a large number of tests, in which the amounts of carbon dioxide liberated were varied from 2.5 to 13 milligrams, proved the apparatus to be 100 per cent efficient under the experimental conditions.

RESULTS AND DISCUSSION

Presentation of data

The results obtained in the various experiments are summarized in tables 1 to 3, inclusive, and they are shown graphically in figures 2 to 7, inclusive. For each of the processes considered, the data are presented as percentage differences between the values for the controls and those for the irradiated seedlings.

TABLE I
Relative radiosensitivity of the several organs of seedlings at various ages and maintained at different temperatures

ORGAN	MAINTAINED AT 26°C.			MAINTAINED AT 19.5°C.	
	30 hours	52 hours	76 hours	72 hours	94 hours
Coleoptile	1.0	1.0	1.0	1.0	1.0
Leaf	1.0	1.4	1.4	1.5	1.4
Primary root	1.7	1.5	1.4	1.5	1.5
Lateral roots	3.0	1.8	1.6	1.7	1.7

The curves of figures 2 to 5, inclusive, serve to correlate the doses of x-rays administered with the degree of effect produced on the various physiological processes investigated. Since all these curves were derived in a similar manner, it will be sufficient to explain the method of obtaining the data for figure 2, which represents the percentage reduction of the fresh weights of the seedlings, as related to the dosage of x-rays. For each set of plants the difference was computed between the fresh weight of the irradiated seedlings and the corresponding weight of the non-irradiated seedlings; the value thus obtained was divided by the fresh weight of the non-irradiated seedlings, the result indicating the percentage reduction in fresh weight of the seedlings brought about by exposure to x-rays. A statistical analysis of the data showed that the results were significant.

Before discussing the results in detail, it may be well to point out that the effects produced by x-rays on the different physiological processes in-

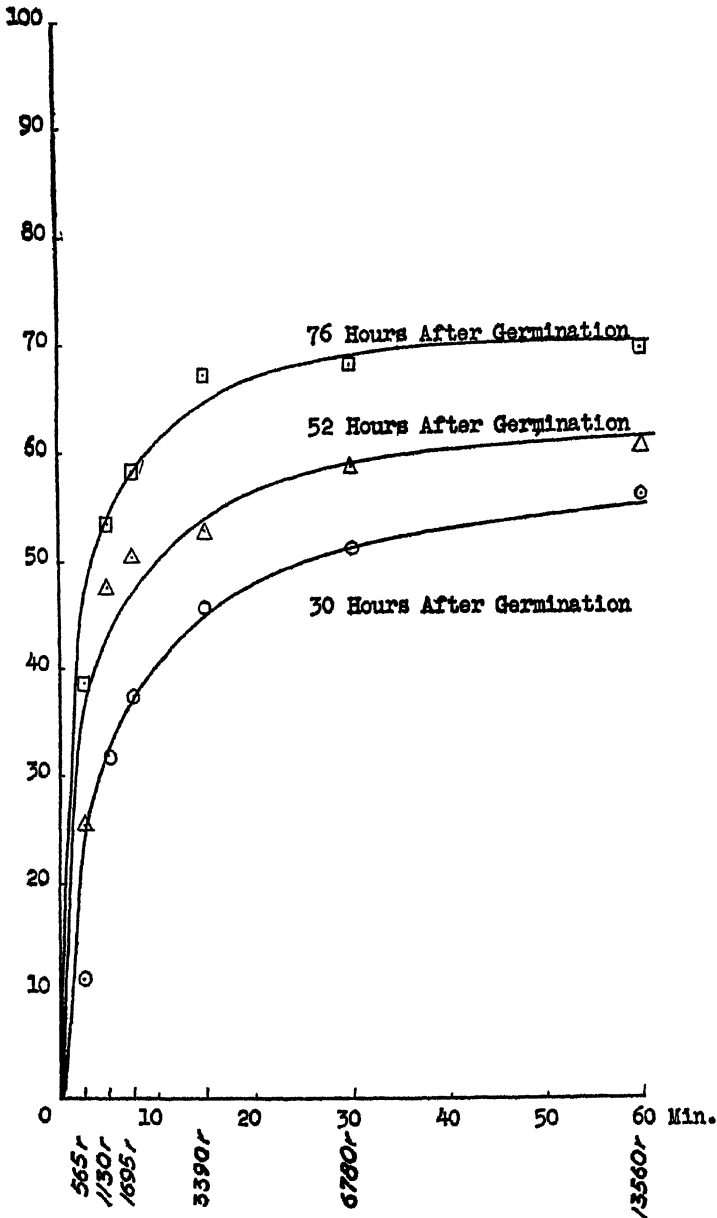


Fig. 2. Percentage reduction of fresh weights of seedlings for the different intervals of measurement. The ordinates represent percentage reduction; the abscissas represent dosage—the upper line being expressed in minutes of exposure, and the lower line giving the number of roentgens.

vestigated were, with one exception, all inhibitory. Growth, as measured by several criteria, and respiration were checked after irradiation of the seedlings. The one exception noted was in a transitory acceleration of respiration in seedlings which had received a light dosage of irradiation.

Throughout the discussion which follows, it should be borne in mind that the statements made and the conclusions reached are applicable only for the range of x-ray exposures investigated in these experiments, and that the effects observed are described with reference to their manifestation in the responses of the very young wheat seedlings employed in this study.

Influence of x-rays on weights of seedlings

Upon examining the curves of figure 2, it is obvious that in all cases a retardation of fresh weight was brought about by the radiation. The retardation becomes progressively greater as the dosage of x-rays is increased. Retardation, however, is not proportional to the amount of radiation received by the seedlings. Light dosages have a very marked effect. As the dosage is increased, the curve in each case rises more and more gradually, so that it tends to become horizontal. There is little further retardation when the dosage is increased beyond 3390 roentgens (15 minutes' exposure). The general form of the curve is the same for the seedlings that were weighed at an interval thirty hours after germination (five to six hours after the administration of x-rays) as for those weighed seventy-six hours after germination (fifty-two hours after irradiation). The curve for growth retardation, however, rises more rapidly and to a greater height with an increase in the time before measurements are made. Growth retardation therefore becomes more pronounced with lapse of time.

Figure 3 indicates the manner in which the dry weights of seedlings are influenced by longer and, therefore, larger doses of x-rays. As might be expected, the curves of figure 3 bear a close resemblance to those of figure 2. The application of x-rays caused a marked retardation in the accumulation of dry material in the growing parts of the irradiated seedlings. With increase in dosage the effect, like that on fresh weight, increases rapidly at first and then more and more slowly; beyond about 3390 roentgens the effect remains nearly constant. The three curves, representing determinations made thirty, fifty-two, and seventy-six hours after germination, are characterized by a general similarity in shape. They resemble in form those for fresh weights and, like the latter, illustrate the fact that the effect of radiation becomes more pronounced as the seedlings grow older.

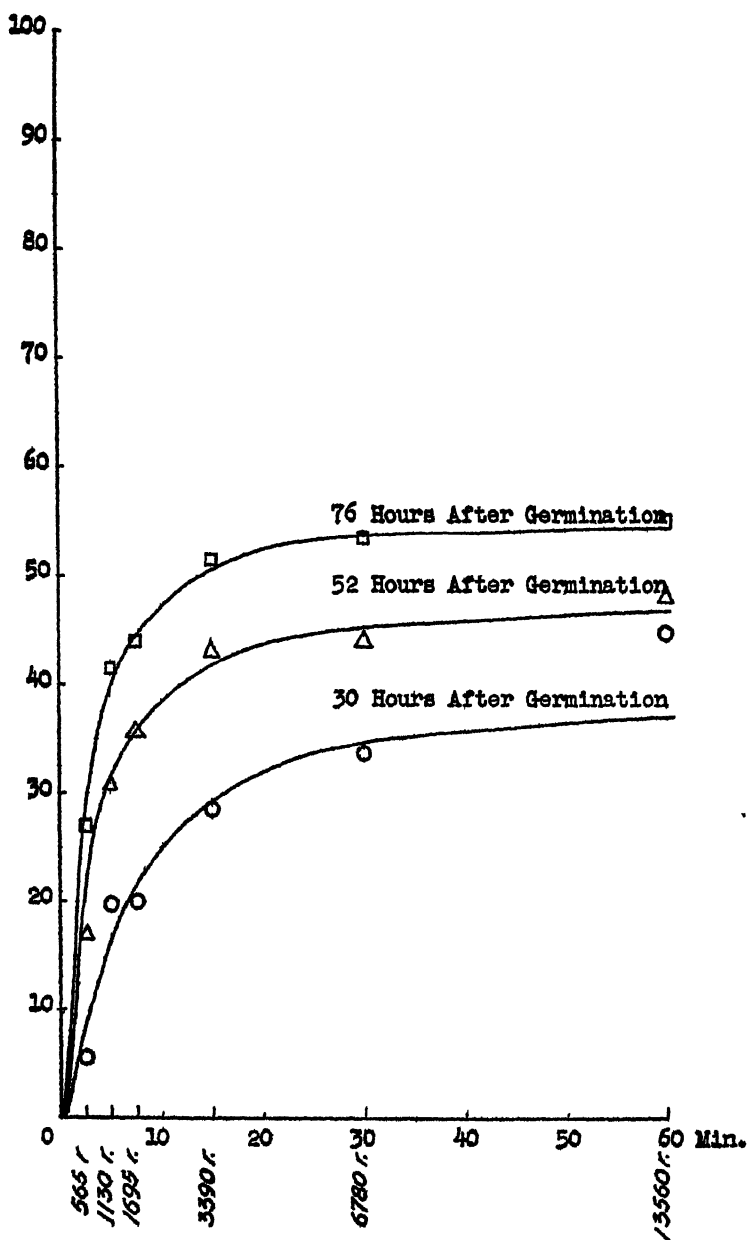


Fig. 3. Percentage reduction of dry weights of seedlings for the different intervals of measurement. The ordinates represent percentage reduction; the abscissas represent dosage—the upper line being expressed in minutes of exposure, and the lower line giving the number of roentgens.

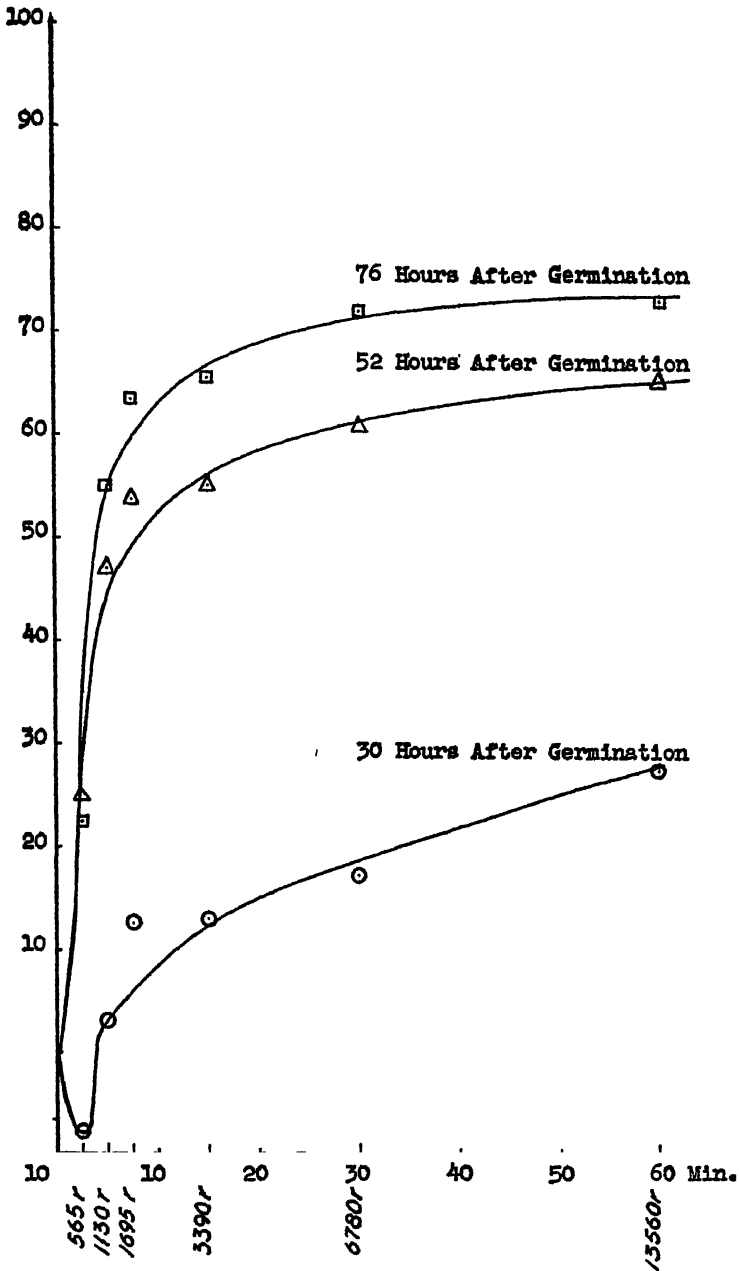


Fig. 4. Percentage reduction in carbon dioxide production of seedlings for the different intervals of measurement. The ordinates represent percentage reduction; the abscissas represent dosage—the upper line being expressed in minutes of exposure, and the lower line giving the number of roentgens.

Influence of x-rays on carbon dioxide production of seedlings

Inspection of the curves of figure 4 shows that, in general, the application of x-rays retarded the rate of production of carbon dioxide by the seedlings. The retarding effect increased with increasing dosages of x-rays. Thus the general form of the curves representing respiration agrees with that of the curves for the reduction of fresh and dry weight.

The most striking feature of these graphs is the temporary acceleration in respiration of seedlings that had been exposed to 565 roentgens. This acceleration is denoted by a fall in the curve representing retardation of respiration. The acceleration occurred in measurements made thirty hours after germination, or six hours after exposure to the radiation. It is interesting to note in this connection that many investigators have reported a temporary increase in respiratory activity, often accompanied by a rise in temperature, following injury of plant parts by wounding. Thus Richards (1896, 1897), Magness (1920), Johnstone (1925), Lutman (1926), and Coleman, Rothgeb, and Fellows (1928) are among those who have found a marked increase in the evolution of carbon dioxide from injured tissues. This accelerated production of carbon dioxide has been shown to be due partially to facilitation of gaseous exchange between the tissues and the surrounding atmosphere. Acceleration of chemical changes, especially those leading to sugar accumulation and increase in oxidizing and diastatic enzymes, has also been shown to play a part in the observed increase in respiratory activity. It seems likely that the temporary acceleration of respiration found in the present study may have been related to the many cases in which similar acceleration follows wounding injury of tissues.

The increased respiration noted in the one case just discussed was followed by subnormal rates. For later periods of observation and for doses ranging from 1130 to 13,560 roentgens at the thirty-hour interval, carbon dioxide production by the irradiated seedlings was always retarded. From the curves of figure 4 it may be seen that, as the x-ray dosage is increased, the retardation of respiration increases rapidly at first; beyond 3390 roentgens, however, it increases very slowly. In general form these curves show a marked agreement with those for fresh and dry weights. Also, as was true in the case of the weights, the degree of retardation was enhanced by the lapse of time before the measurements were made.

Influence of x-rays on the linear growth of the four organs of seedlings

General influence. The four curves of figure 5 indicate the way in which growth retardation of the four organs of the wheat seedlings is related to increasing doses of x-rays. These curves are based upon measurements of

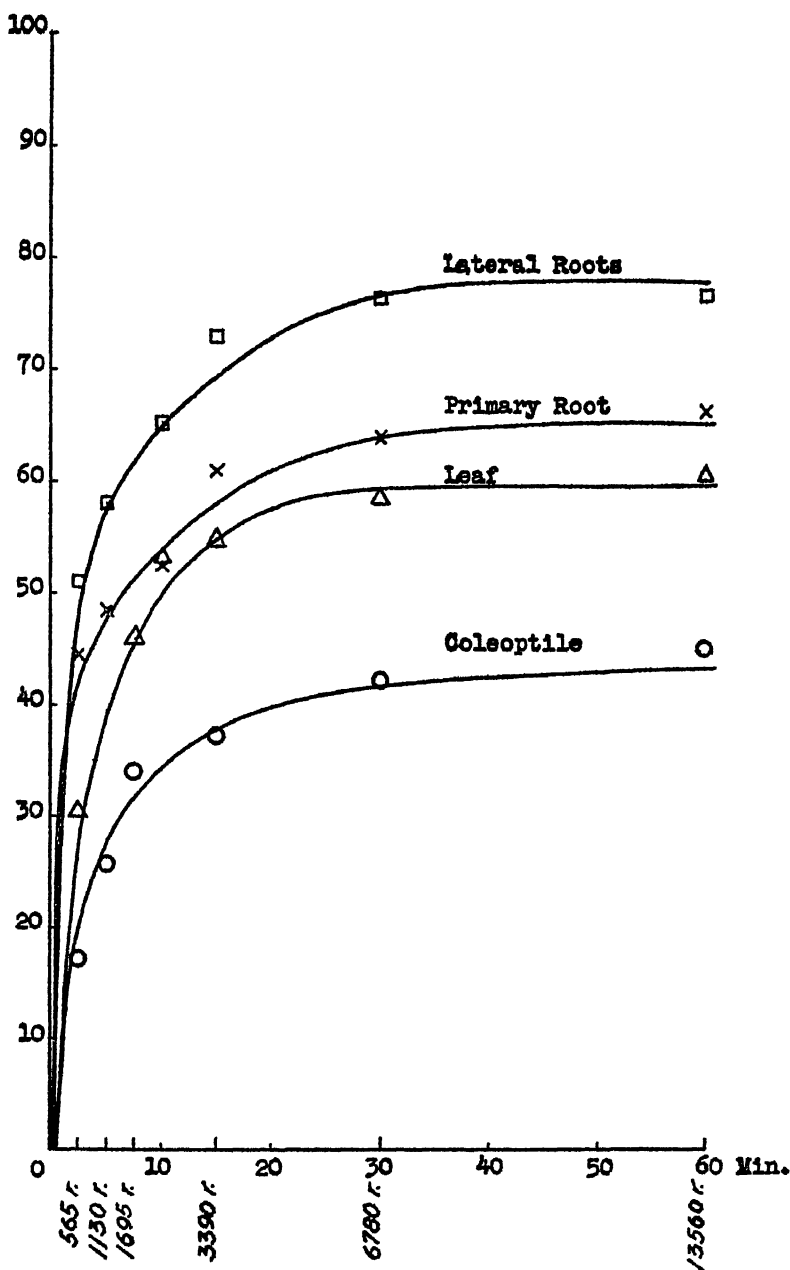


Fig. 5. Percentage reduction in linear growth of the four organs of the seedlings when measured 52 hours after germination began. The ordinates represent percentage reduction; the abscissas represent dosage—the upper line being expressed in minutes of exposure, and the lower line giving the number of roentgens.

the lengths attained by the growing portions of the seedlings fifty-two hours after germination began, or twenty-eight hours after radiation was administered. The upward slope of the curves for all organs (lateral roots, primary root, leaf, and coleoptile) shows that greater doses of x-rays produced increased retardation in the linear growth of these organs. The increase in retardation is rapid at first; but beyond 3390 roentgens the curves rise very gradually. It is clear that in form these curves resemble closely those derived from the measurements of fresh weights, dry weights, and respiration.

Differences in radiosensitivity of the four organs. In the region beyond 3390 roentgens, the four curves of figure 5 appear to run approximately parallel to one another. It may be noted that, for any given dosage, the greatest growth-retarding effect is produced on the lateral roots; less effect is produced on the primary root, still less on the leaf, and least on the coleoptile. It seems clear, therefore, that the four organs have different degrees of radiosensitivity, the lateral roots being most sensitive and the coleoptile least sensitive. Instead of being truly parallel to one another, the smoothed curves probably bear a constant relation to one another—namely, a relation such that the ordinates of one curve may be derived by multiplying the ordinates of another curve by a constant factor. This constant relationship among the curves seems to hold approximately true, at least for doses greater than 3390 roentgens. Thus the relative degrees of radiosensitivity of the four organs appear to be independent of the magnitude of the x-ray dose, at least beyond a certain value (3390 roentgens).

To test the constancy of this relationship, calculations of the relative degrees of radiosensitivity of the four organs were made from values taken from the graphs of figure 5. Since the coleoptile is the least sensitive organ, it was assigned a radiosensitivity value of unity. For a given abscissa (or dosage), the ordinates (or percentages of reduction) for the lateral roots, the primary root, and the leaf were divided by the ordinate (or percentage reduction) for the coleoptile. The quotients obtained indicate the different degrees of radiosensitivity of the four organs to the dose in question. It is found that the values for the radiosensitivity of a given organ are almost constant for doses above 3390 roentgens. Within the range considered, radiosensitivity therefore appears to be independent of dosage. But it is different for each of the organs. For the coleoptile the average index of radiosensitivity is 1.0; for the leaf, 1.4; for the primary root, 1.5; and for the lateral roots, 1.8.

Effect of time of observation on radiosensitivity. It should be noted at this point that the values for radiosensitivity discussed so far were based upon measurements of the different organs fifty-two hours after germination be-

gan. It is important now to consider what influence the time of measurement may have on these values. Information concerning this question may be obtained by comparing the data based upon measurements of linear growth that were made thirty hours, fifty-two hours, and seventy-six hours after germination began. Since all seedlings were irradiated twenty-four hours after germination commenced, the respective intervals between the time of irradiation and the time of measurement were six, twenty-eight, and fifty-two hours. In considering these results, it should be borne in mind that considerable experimental error may be present in the data secured thirty hours after germination started, owing to the shortness of the organs measured.

Curves plotted to show growth retardation for the thirty-hour and the seventy-six-hour periods exhibit the same general characteristics as those of figure 5, which already have been discussed in detail. Certain differences, however, appear when the curves for these three intervals are compared. The coleoptile and leaf curves practically coincide for the thirty-hour interval, while the leaf and primary-root curves practically coincide for the seventy-six-hour interval. It is difficult to say definitely whether the differences in the three sets of data are really significant or whether they result from experimental error.

The coincidence of the leaf and coleoptile curves for the thirty-hour interval may have resulted from the difficulty of measuring these organs accurately when they were very short and barely, if at all, distinguishable. Although clear differences in radiosensitivity between the leaf and the coleoptile were apparent fifty-two and seventy-six hours after germination commenced, it seems likely that failure to note them at the end of the thirty-hour period was due to the difficulty just mentioned. Furthermore, the coincidence of the leaf and the primary-root curves after seventy-six hours might be attributable to experimental error. By reference to figure 5 and table 1, it will be seen that fifty-two hours after germination the radiosensitivity of the leaf was not very different from that of the primary root (1.4 and 1.5 respectively).

Average values for the relative degrees of radiosensitivity of the four organs for each of the three observation periods are summarized in table 1. The corresponding averages for the fifty-two-hour interval and the seventy-six-hour period agree fairly closely with one another, but they differ from those for the thirty-hour interval. In view of the large experimental error involved in measurements made at the thirty-hour interval, however, the differences which are recorded should probably not be regarded as significant. Accordingly, it seems safe to conclude that the relative degrees of radiosensitivity of the four organs are essentially independ-

ent of the time of observation and the magnitude of the dose administered, within the limits of the experimental conditions involved.

Effect of dose on radiosensitivity. It has been shown in the preceding sections of this paper that the relative degrees of radiosensitivity of the four organs appear to be independent of the magnitude of the x-ray dose administered—within the range of dosages investigated in the present study. As previously pointed out, this generalization appears to hold definitely for the higher portion of the dosage range tested—namely, 3390 to 13,560 roentgens.

It is of special interest to inquire whether this relation exists, even approximately, within a very low range of x-ray dosage. Information bearing on this question is available in a paper by Failla and Henshaw (1931), which reports results obtained with doses ranging from 0 to 1400 roentgens. Figure 6 shows these data plotted in the same manner as the results secured in the present study. A comparison of figure 6 with figure 5 reveals certain differences in the forms and positions of the curves for the corresponding organs. In particular, the curves for the primary and the lateral roots of figure 6 cross twice within the range of 0 to 1400 roentgens, while the curves for these organs do not cross in figure 5; in fact, they remain a considerable distance apart throughout the higher range of x-ray dosages. It is evident also that with the smaller doses represented in figure 6 (0 to 1400 roentgens), there is no constant relationship among the curves for the four organs, such as appears in these curves in figure 5. In the lower range of x-ray dosages, therefore, the relative degree of radiosensitivity of the four organs varies with the magnitude of the dosage.

Effect of temperature on radiosensitivity. In the experiments of Failla and Henshaw (1931) the seedlings were grown at a temperature of 19.5°C., whereas, in experiments described in this paper, the temperature was maintained at 26°C. In order to ascertain whether the differences between the two sets of curves (figure 6 and figure 5) might be due to differences in temperature, several experiments were performed at 19.5°C. The seedlings were exposed to the same doses of x-rays as those employed in the growth tests at 26°C.—namely, 565 to 13,560 roentgens. It is worthy of note that measurements made seventy-two hours after germination began (figure 7) on the seedlings grown at 19.5°C. yielded similar results to those obtained from seedlings maintained at 26°C. Likewise, data obtained ninety-four hours after germination commenced resulted in analogous curves. It seems probable, therefore, that the differences between the results described in this paper and those reported by Failla and Henshaw are not caused by differences in temperature.

The measurements obtained at 26°C. for the seventy-six-hour interval

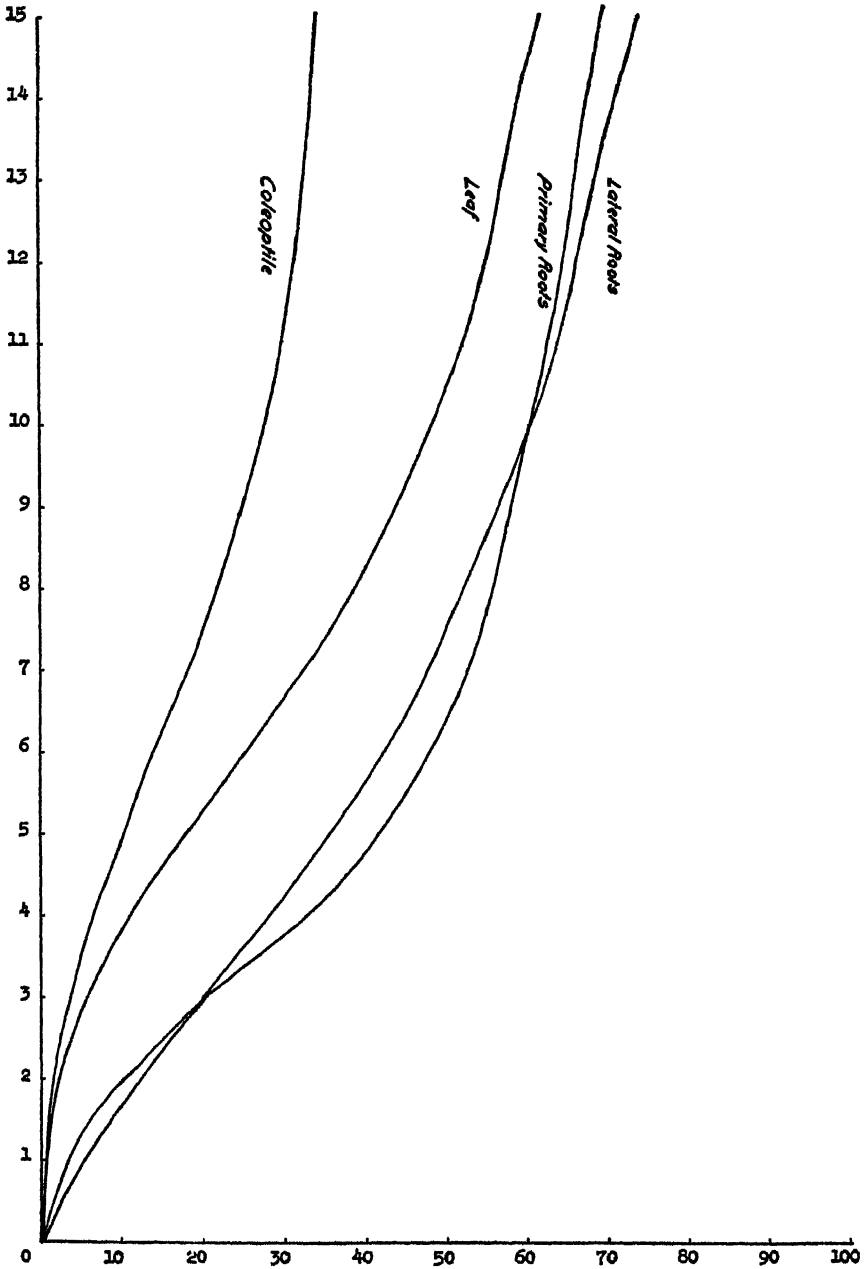


Fig. 6. Percentage reduction in linear growth of the four organs of the seedlings when measured 94 hours after germination began (taken from the data of Failla and Henshaw, 1931). The ordinates represent dosage in roentgens $\times 100$; the abscissas represent percentage reduction.

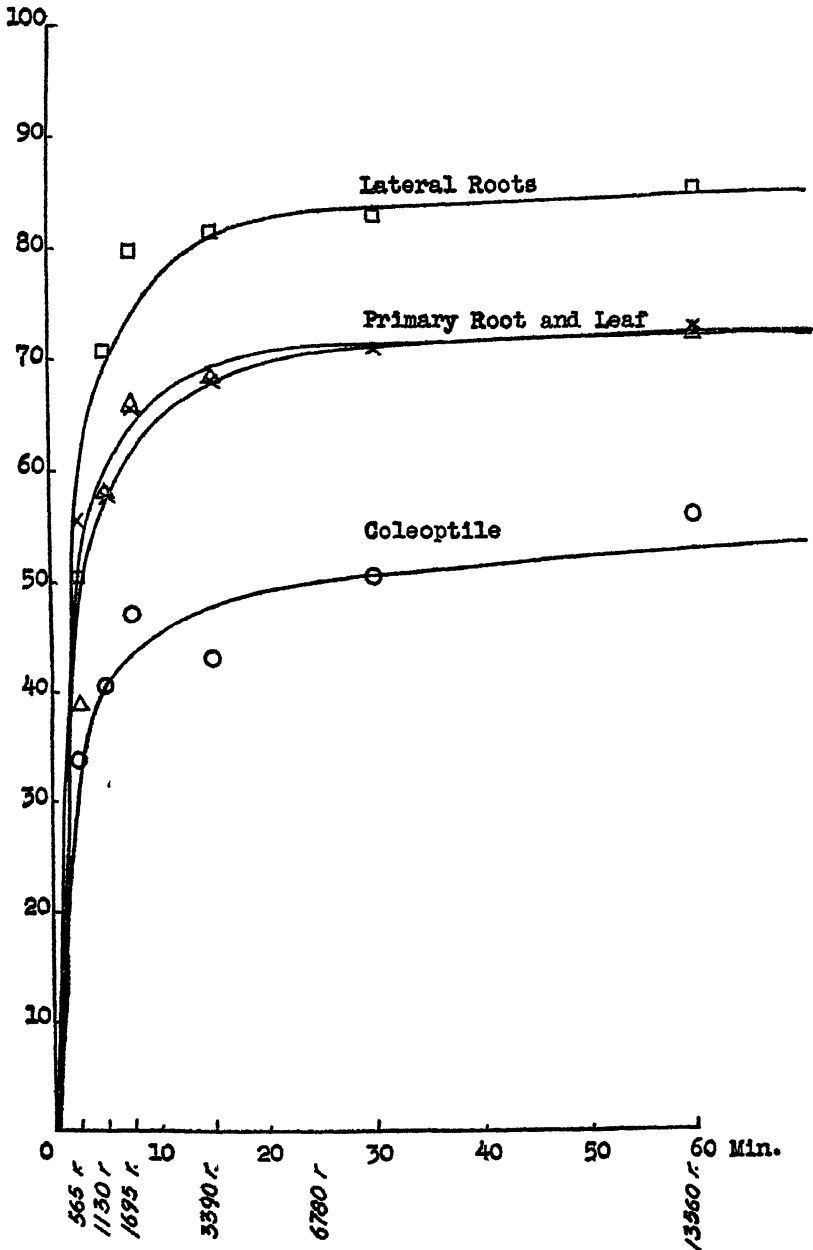


Fig. 7. Percentage reduction in linear growth of the four organs of the seedlings when measured 72 hours after germination began, when the temperature was varied to 19.5°C. The ordinates represent percentage reduction; the abscissas represent dosage—the upper line being expressed in minutes of exposure, and the lower line giving the number of roentgens.

manifest a somewhat greater response of the seedlings to radiation than do those at 19.5°C. for the seventy-two-hour period. This may be explained by the fact that the control seedlings attain a greater growth at the higher temperature than at the lower temperature. In view of these results, it is to be expected that the relative degrees of radiosensitivity of these organs should be approximately the same at 19.5°C. as at 26°C. (table 1). And these degrees of radiosensitivity are in rather close agreement for all of the time periods in question—seventy-two and ninety-four hours after germination began in the 19.5°C. experiments, and fifty-two and seventy-six hours after germination started in the 26°C. experiments. It appears, therefore, that the relative radiosensitivity of the four organs is independent of the temperature within the range from 19.5°C. to 26°C.

General relation between dosage and physiological response

At this point it seems worth while to consider the general similarity in form of the curves for all of the different physiological processes shown in figures 2 to 5, inclusive. All of these curves follow the same general course. With increasing dosage of x-rays the curve representing the response in every case rises rapidly at first, then more and more gradually, until it finally becomes practically horizontal. The region of rapid rise includes dosages from 0 to about 3390 roentgens. From 3390 to 13,560 roentgens there is little further increase in the effect upon any of the physiological processes. These curves showing relations between dosage and retardation of physiological processes resemble many curves which have been obtained by other workers in studies correlating stimulus and response. It seems to be a rather general rule that with increase in the amount of stimulus, the response increases rapidly at first and then more and more slowly, until finally further increase in stimulus results in no increase in response.

One hypothesis to account for the decreasing influence of greater and greater doses of x-rays (beyond 3390 roentgens) may be based upon the supposition that the population of cells making up each organ is composed of individual cells that vary in radiosensitivity. The individual cells may be assumed to exhibit a wide range of sensitivity, from those which are highly sensitive to those which are only slightly sensitive. This gradation in radiosensitivity may depend in part, at least, upon the phase of development of the cells; rapidly dividing cells probably are most susceptible to radiation (law of Bergonié and Tribondeau, 1906). The most sensitive cells would be influenced by the small doses of x-rays, but the most resistant ones would not be affected appreciably even by the largest doses employed in these experiments. According to this view, the rapid initial rise

of the curves of figures 2 to 5 indicates that most of the cells in each of these populations are markedly affected by doses smaller than 2260 or 3390 roentgens. The other cells are not markedly affected by doses four times as great. In all cases some growth takes place, even with the largest doses employed. Great emphasis should be placed upon the fact that it is difficult to bring about complete cessation of growth and respiration by means of x-ray treatment. This is in marked contrast to the ease with which all vital activity of seedlings may be inhibited subsequent to the application of chemical poisons.

It would be of interest in this connection to correlate growth retardation with anatomical and cytological features of the plant organs in question. Detailed observations, however, are not available at present for an interpretation along these lines. Rapid cell division in the root is largely confined to a very short apical region, and most of the elongation of the root occurs in a short zone behind the region of cell division. According to Tetley and Priestley (1927), cell division in the coleoptile ceases at a very early stage. They state that if the coleoptile of a wheat grain that has been soaked in water for a few hours is removed and sectioned, all the regions of the coleoptile except the vascular strands will be found to be vacuolating. These investigators were not able to detect cell divisions in the tissues of the coleoptile. Growth of this organ depends almost entirely upon the elongation of cells in a zone lying a short distance below the apex. The fact that cell division in the coleoptile ceases at such an early age may account for its low radiosensitivity. Growth of the leaf is brought about largely by cell division and enlargement in a zone near the base of the leaf.

It is reasonable to expect that a retardation in the accumulation of fresh and dry materials and in the production of carbon dioxide would accompany a retardation in the rate of linear growth of the organisms. Therefore, the similarity of the effects of x-rays on the different processes investigated would be expected.

Correlations between different responses to radiation

Certain interesting relations between different responses to radiation may be brought out by determining the ratios existing between corresponding sets of data for any two physiological processes. For example, the amount of carbon dioxide produced by seedlings which had received a given dose of x-rays may be divided by the value of the fresh weight for seedlings exposed to the same dose. Similar ratios may be secured for each interval of measurement. From this procedure it is possible to ascertain the exact quantity of carbon dioxide liberated by a given set of seedlings per gram of fresh weight per hour.

The experimental data for the other physiological processes investigated were correlated in a manner similar to that just described. A summary of the results is presented in table 2. In this table one column contains the values obtained for non-irradiated seedlings, while the other column includes the averages of ratios determined for irradiated seedlings.

A supplementary means of correlating the experimental results is afforded by epitomizing the data in tabular form, as in table 3. In this table

TABLE 2
Summary of correlations of experimental data

RATIO	MEASURING INTERVAL (hours)	CONTROL SEEDLINGS	IRRADIATED SEEDLINGS
Mg. CO ₂ per gram fresh weight per hour	30	3.01	4.51
	52	1.99	1.90
	76	1.04	1.03
Mg. CO ₂ per gram dry weight per hour	30	30.6	36.7
	52	19.6	14.9
	76	13.0	9.5
Mg. fresh weight per mm. aver- age linear growth	30	12.6	10.9
	52	116.5	131.2
	76	132.8	178.7
Mg. dry weight per mm. aver- age linear growth	30	12.4	13.2
	52	11.8	16.7
	76	10.7	19.7
Mg. fresh weight per mg. dry weight	30	10.1	8.2
	52	9.9	7.9
	76	12.5	9.2
Mg. CO ₂ per mm. average lin- ear growth per hour	30	.38	.49
	52	.23	.25
	76	.14	.18

the percentage reduction obtained for each exposure is presented for each method of measuring the effects of radiation on the wheat seedlings and for all intervals of measurement. From the data thus arranged, a direct comparison may be made between any two responses of the seedlings to x-rays.

Carbon dioxide production and weights. Inspection of table 3 shows that for all exposures when measurements are made thirty hours after germination began, the amount of fresh weight is reduced to a greater extent than is the carbon dioxide production. Reference to table 2 shows that at the thirty-hour interval the rate at which carbon dioxide is produced by the irradiated seedlings is more rapid than that for the non-irradiated seed-

lings of the same fresh weight. It is evident, therefore, that the effect of radiation is to accelerate the production of carbon dioxide per unit of fresh weight at this period.

It will be seen that for periods longer than thirty hours after germination, the total fresh weight is reduced approximately to the same extent as is the amount of carbon dioxide production, following irradiation. From these considerations it appears that while x-rays retard the growth of the

TABLE 3
Percentages of reduction obtained at different intervals for various methods of measurement

MINUTES' EXPOSURE	FRESH WEIGHT	DRY WEIGHT	CARBON DIOXIDE PRODUCTION	LINEAR GROWTH
30 hours after germination				
2.5	11.1	5.6	-7.7	15.7
5.0	31.8	19.8	3.1	22.8
7.5	37.5	20.0	12.6	28.9
15.0	45.7	28.7	12.9	30.0
30.0	51.1	33.7	17.2	36.9
60.0	55.7	44.7	27.4	44.1
52 hours after germination				
2.5	25.4	17.1	25.2	39.9
5.0	47.7	30.9	47.3	47.3
7.5	50.3	35.7	53.7	53.4
15.0	52.8	43.2	55.2	59.7
30.0	58.6	44.3	60.8	63.4
60.0	60.2	48.2	65.3	65.3
76 hours after germination				
2.5	38.6	27.1	22.5	48.5
5.0	53.5	41.4	55.0	65.6
7.5	58.2	44.0	63.2	70.6
15.0	67.2	51.5	65.4	74.8
30.0	67.9	53.4	71.9	76.4
60.0	69.2	54.6	73.2	78.7

seedlings in mass, they bring about an equivalent reduction in the production of carbon dioxide. Thus, the metabolic activity of the irradiated organs, mass for mass, remains essentially the same as that for the non-irradiated organs, at the later periods of measurement, and for the amount of radiation administered in this investigation.

If, on the other hand, dry weights are correlated with the carbon dioxide production, there is a greater reduction in carbon dioxide production than in the total dry weight, except thirty hours after germination, when the reverse is true. Thus, at the later intervals of measurement less carbon

dioxide is produced by the irradiated seedlings than by the non-irradiated seedlings of a given dry weight. It is evident, therefore, that one effect of x-rays in the doses administered is to depress the carbon dioxide production per unit of dry weight, except thirty hours after germination, when the effect is to stimulate the respiration per unit of dry weight.

Weight and length. Since it was impossible to weigh the various organs separately, the correlation of weights and lengths has been made on the basis of the average lengths of all four organs (coleoptile, leaf, primary and lateral roots). Comparison of the two sets of data makes it evident that, for a given exposure, there is, in general, a greater percentage reduction in linear growth than in the amount of fresh weight of the irradiated seedlings, except thirty hours after germination. Thus, for the same fresh weight, the average length of the four organs is less in the irradiated seedlings than in the controls. In other words, the irradiated organs are somewhat heavier per average unit length than the non-irradiated organs. These relations between weight and length do not apply to seedlings measured five to six hours after the administration of x-rays, or thirty hours after the beginning of germination. At this stage the fresh weight seems to be more retarded than the linear growth of the irradiated seedlings. This effect probably is not very significant, owing to the shortness of the organs and the correspondingly large experimental error involved in measurements made at this period.

When dry weights are correlated with the average lengths of the organs, it is evident that there is less reduction in the dry weights than in the average linear growth of the irradiated seedlings. Accordingly, an effect of x-rays in the doses administered is a stunting of linear growth, accompanied by a slight increase in the average diameter of the organs. The differences in diameter were noticeable during the course of the experiments, although they were not measured.

Water content. When the dry weights of seedlings are correlated with the fresh weights, it is found that the irradiated seedlings have lower fresh weights than the non-irradiated seedlings of the same dry weight. In other words, there is a greater percentage reduction in fresh weight than in dry weight in the irradiated seedlings. Hence, the irradiated seedlings contain less water than the non-irradiated seedlings of the same fresh weight. This statement is true for all intervals of measurement and for all doses of x-rays administered.

The effect of x-rays in producing a relative reduction in water content of the organs of the wheat seedlings is probably due to decreased imbibitional or osmotic powers of the irradiated organs.

Linear growth and carbon dioxide production. If the carbon dioxide pro-

duction of the seedlings is correlated with the average linear growth of the four organs, it is apparent that for a given reduction in carbon dioxide production, there is, in general, a greater reduction in the average linear growth of the irradiated organs. This is not so obvious at the fifty-two-hour interval as at the other two periods of measurement. Generally speaking, however, the irradiated seedlings respire at a more rapid rate than the non-irradiated seedlings of the same average length. Thus, x-rays, in the doses administered, increase the rate of respiration per unit length of the organs.

General correlations between physiological responses to x-rays. When a general survey is made of table 3, two facts of interest become evident. In the first place, the percentage reduction obtained in a particular response to a given dose of x-rays is greater with an increasing interval before measurement of the effect. For example, at the age of thirty hours the percentage reduction in linear growth of the seedlings which received sixty minutes of treatment is 44; at the age of fifty-two hours it is 65; and at seventy-six hours, 79. It is clear, therefore, that there is a latent period in the biological action of radiation, whereby the observable effect becomes more marked with the lapse of time between irradiation and measurement of the response. It is impossible to say that the maximum effect was obtained with the time intervals used in the present experiments. If observations had been made at longer intervals, it is probable that still more marked effects would have been found.

In the second place, there is a close correlation among the percentages of reduction obtained for fresh weights, carbon dioxide production, and linear growth, as measured at the later periods. From this fact it appears that any one of these effects might be taken as a criterion of general injury to the organism, for certain practical purposes.

Apparent correlation between radiation effects and aging. Several investigators have obtained evidence which indicates that the application of x-rays causes modifications of the irradiated material that are associated with senescence. Isaacs (1932) was of the opinion that x-rays speed up the life processes in the blood cells, thus hastening the advent of old age. Nemenow (1925), citing the results of a number of investigators who had studied the action of x-rays on living materials, called attention to the fact that the application of x-rays brought about changes which were characteristic of those accompanying old age.

In view of these observations, it seemed of interest to analyze the data obtained from the present experiments to see whether a similar conclusion might be drawn from them. The values presented in table 2 serve as a basis for this analysis. Thus, at the thirty-hour age the amount of carbon di-

oxide liberated per hour per gram of fresh weight for the non-irradiated seedlings is 3.01 milligrams. At the fifty-two-hour age the corresponding index for the non-irradiated seedlings is 1.99 milligrams per hour per gram. Similarly, at the seventy-six-hour age the respiratory index has a value of 1.04 milligrams per hour per gram. Hence, with increasing age, the rate of respiration per gram of fresh weight decreases in the control seedlings.

If the irradiated seedlings had been so modified as to manifest characteristics associated with senescence, it follows that the respiratory index should fall below that which is characteristic of the non-irradiated seedlings of the same age. It is demonstrated clearly in table 2 that there is no significant difference between the values of the respiratory index for the irradiated seedlings and those for the controls in the case of measurements made at the later intervals after germination began. On the contrary, the respiratory index of the irradiated seedlings which were measured at the thirty-hour interval is higher than the index for the non-irradiated seedlings of the same age. Hence, five or six hours after irradiation the x-rays have affected the seedlings in such a manner as to change the index to that characteristic of non-irradiated seedlings of younger age. Accordingly, it may be concluded that the effect of x-rays on the growing parts of the wheat seedlings is a stimulation of respiratory activity, followed later by a return to normal activity. The evidence obtained from the respiratory index, then, does not indicate the advent of premature senescence in the irradiated seedlings. It suggests, rather, a temporary setback, followed by the resumption of behavior characteristic of normal seedlings of the same age.

The remaining experimental data have been considered with reference to their significance in demonstrating premature senescence in irradiated seedlings. Correlations of carbon dioxide production with dry weight at the later measuring intervals suggest premature aging in the irradiated seedlings. Likewise, the values obtained for the ratios of fresh weight to linear growth at the later intervals are characteristic of senescence in irradiated seedlings. In general, however, the irradiated seedlings appear to be stunted, rather than advanced in age. Thus, the evidence obtained from the present investigation offers no conclusive corroboration of the theory that x-rays induce premature senescence.

SUMMARY

This paper presents the results of an experimental study of the influence of x-rays on the growth and respiration of very young wheat seedlings. After being soaked in water for three hours, the seeds were planted on moist filter paper and left to germinate. At the end of twenty-four

hours the seedlings were irradiated with doses of x-rays ranging from 565 to 13,560 roentgens. Periodic determinations were then made of respiratory activity and growth during the first hundred hours of seedling development. The main results of this study are given below. The conclusions drawn from these experiments can be regarded as applicable only for the range of x-ray exposures used, the time intervals involved, and the particular biological material investigated in this work.

1. The effects of x-rays on wheat seedlings were found to depend upon (a) the dose of radiation administered, (b) the time when the effect is observed, and (c) the type of response considered.

2. Retardation of both fresh-weight and dry-weight production by the growing parts of the seedlings was brought about by all doses of x-rays employed, and this retardation became progressively greater with lapse of time and with increase in dosage.

3. Respiration was depressed in all cases except those in which seedlings were tested five or six hours after irradiation (i.e., thirty hours after the beginning of germination). At that time the seedlings which had received the smallest dose of radiation (565 roentgens) exhibited an accelerated rate of respiration.

4. Retardation of linear growth of the coleoptile, the leaf, the primary root, and the lateral roots was obtained with all doses and time intervals employed.

5. Each of the organs investigated seemed to have a characteristic degree of radiosensitivity. The lateral roots were most sensitive and the coleoptile was least sensitive. The relative degrees of radiosensitivity of the four organs studied appeared to be essentially independent of time of observation, of dosage, and of temperature—within the particular ranges of these conditions investigated.

6. Certain correlations between different responses to radiation were noted, as follows:

- (a) X-rays did not affect the rate of carbon dioxide production per unit of fresh weight when the seedlings were more than fifty-two hours old. They increased the value of this quotient thirty hours after germination (five or six hours after irradiation).

- (b) The rate of carbon dioxide production per unit of dry weight was lower in the irradiated seedlings than in the non-irradiated seedlings, fifty-two or more hours after germination began. This ratio was greater in the irradiated seedlings thirty hours after germination.

- (c) Stunting of linear growth was accompanied by a reduction in water content and a slight increase in weight per unit length of the organs in irradiated seedlings.

(d) The irradiated organs respired at a more rapid rate than the non-irradiated ones of the same average length.

In all these cases the effect was more apparent, the larger the dose and the longer the interval before measurements were made.

7. The data presented were analyzed to determine their bearing on the problem of aging as manifested in the experimental material. In view of the conflicting evidence obtained from these data, it was suggested that the theory that irradiation induces premature aging was not corroborated by the present experiments.

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The relation of *Uromyces Caladii* and other rusts to their hosts

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(WITH PLATES 6-8)

Dufrenoy (1928, 1929) in a series of articles upon relations between seventeen plant parasites and their hosts, reaches conclusions as to the effect of the haustoria on the host cell quite the opposite to those which I have stated in connection with my own observations upon the rust parasites. The following haustorial parasites were studied by Dufrenoy: *Puccinia Sorghi*, *Puccinia Asphodeli*, *Uromyces Caladii*, *Peronospora Schleidenii*, *Phytophthora infestans*, *P. terrestris*, *Blepharospora cambiovora* and *Fusarium Rubi*. Later (1930), he adds observations upon *Helminthosporium* in barley and *Pseudoperonospora humuli*.

Dufrenoy (1928) thinks that the finely vacuolate condition of the protoplasm which is, at times, found in healthy cells in periods of metabolic activity is brought about by a return from the univacuolate condition of more mature cells to the more foamy condition of embryonic cells and he interprets this as a fragmentation of the vacuolar system effecting an increase of contact surfaces between cytoplasm and vacuoles. Dufrenoy finds in cells infected by fungous parasites, instead of the normal central vacuole, a system of small vacuoles lying in a network of cytoplasmic strands. He thinks this condition is the same in origin and in function as that described for non-infected cells under conditions of metabolic activity. Even more important than this fragmentation of the vacuole he considers the physico-chemical modifications wherein various substances held in solution in the normal vacuole are precipitated as phenolytic compounds in the infected cell. "Plus importantes encore, sans doute, que les modifications morphologiques des vacuome, sont les modifications physico-chimiques de son contenu." (1928).

Dufrenoy's observations of the phenomena of infection are most specifically described in his studies of haustorial parasites. He states (1929) that in the case of *Uromyces Caladii* the entrance of the haustorium causes a condition in the host cell comparable to that described by Magrou (1918) as phagocytosis when orchid cells digest their mycorrhizal invaders. With respect to *Pseudoperonospora humuli* Dufrenoy (1930) compares the condition which follows haustorial entrance to that observed in cells of carnivorous plants by Magenot: "une protéolyse locale, et une fragmentation de l'appareil vacuolaire en un système de petites vacuolaires à contenu riche en protides." He further holds that the condition is advantageous to the haustorial parasite in that it involves an increase of the biochemical reactions and an exaggeration of the processes of disintegration of the al-

bumenoid complexes. "Le suçoir coiffé d'un cytoplasme finement vacuolisé, plonge donc dans un milieu riche en protides solubles."

I have myself found little indication of changes in the host-cell protoplasm around haustoria, but it is entirely possible that an irritation set up by a haustorial invasion might lead to an at least apparent increase of protoplasm in the cell. Such an increase might become visible as a finely vacuolated thickening of the primordial utricle around a haustorium and even over the whole inner surface of the cell. I do not think that this condition need imply the origin of the small vacuoles from a large central vacuole any more than it need imply such an origin for the vacuolization accompanying proteolysis in the uninfected cells to which Dufrenoy refers. Such a vacuolization, instead of being considered a fragmentation of a central vacuole, could be brought into line with the Dangeard and Guilliermond (1929) theory of the origin of vacuoles from small solid "mitochondria-like bodies" by the swelling up and solution of the latter. It may also be noted that precipitation products in vacuoles stained with neutral red are described by Guilliermond as an occurrence in normal cells. He says (1929) that neutral red may thus be considered virtually a specific dye for the vacuome and that as the cell ages it is known that the vacuole shows one of two very different aspects: (1) Numerous thread-like, sticky bodies staining uniformly and deeply with neutral red. (2) Fewer and larger liquid vacuoles in whose paler contents deeply staining precipitates are made to appear through the action of neutral red. Although Dufrenoy found this precipitation only in the vacuoles of infected cells it is to be questioned whether, in view of Guilliermond's work, such a distinction would prove constant.

In all these cases of haustorial parasites Dufrenoy finds that the changes in the vacuoles are preceded and accompanied by plasmolysis of the host cell. "Lorsqu'un filament de rouille pénètre une cellule, la vacuole se rétracte, entraînant la couche du cytoplasme, et la cellule paraît plasmolysée." By cell penetration in this connection Dufrenoy apparently means only the penetration of the cell wall. According to his interpretations of his figures and descriptions of the conditions in *Uromyces Caladii* the haustorium would seem to exert a stimulus upon contact with the plasma membrane which results in excess exosmosis, causing first plasmolysis and then changes in the vacuolar condition. The result is very different, as shown in his figure 1 (1929), from that in my figure 2 of the present article where the contracted cytoplasm is clearly invaginated by a haustorium. In the matter of penetration and of the reaction of the host cell I found that in the corn rust and several other rusts the host cell cytoplasm upon a penetration of a haustorium was invaginated and was not plasmolysed.

The studies of Plowe upon membranes in the plant cell (1930, 1931) give indirect support to the probability of invagination rather than puncturing by haustoria. She emphasizes the elasticity of the plasmalemma by noting that it remains intact about a needle thrust deeply into the protoplasm of an onion cell. Plowe writes: "It is no easy matter to puncture or tear the plasmalemma of the living protoplast. Even a very sharp needle carries a layer of protoplasm with it as it enters the cell: the needle invaginates, rather than pierces the protoplast; the plasmalemma lies next the needle, and the mesoplasm and tonoplast are also indented and carried in."

I found in all the rusts which I have studied that even in cases of extreme and advanced infection there was little change in the appearance of the cytoplasm of the host cells and I concluded (1927), "For the Uredineae in general, I think that an elaborate development of the haustorium has furthered the lack of disturbance in the regions of special metabolism of the host-parasite complex." This same view has recently been supported by Hull (1931) in discussing types of resistance to *Puccinia Sorghi* shown by *Zea Mays*. After describing, for a resistant host, the plasmolysis of both host and parasite cells upon the first formation of primary hyphae, before any haustoria have a chance to form, she notes an exception as follows: "When large substomatal vesicles are formed in the resistant host the fungus is able to proceed with its life-cycle up to the period of sporulation. Growth is more rapid than in the susceptible host and haustorial contacts are made with host cells before the latter have time to establish any antagonistic response. *Once this contact is complete there is a perfect equilibrium between host and fungus and henceforth formation of haustoria produces no noticeable reaction in the host cells.*" (Italics are mine.)

I have read with especial interest Dufrenoy's report upon *Uromyces Caladii* because I also have made a study of the host-parasite relations of this rust. Dufrenoy in his first article (1928) writes that in the infected cells the vacuole fragments in such a way as to surround an haustorium with an aureole of small vacuoles and that most of the vacuoles in the vicinity of haustoria form, with neutral red, precipitations applied to the surface of the vacuoles until there is no color left within the vacuole. Then "La cellule d'ailleurs perd sa turgescence. Le cytoplasme, qui se détache de la paroi cellulaire prend les aspects que les descriptions de Guilliermond ont rendu classiques pour les cellules plasmolysées." (1928)

In his second article (1929) Dufrenoy describes for the same rust, the fragmentation of the vacuome into a group of small vacuoles "autour du sommet du filament de rouille." It is to be noted that in this description

Dufrenoy uses the term, "filament" instead of the term "suçoir" for the intracellular hypha.

When Dufrenoy speaks of a filament of the rust penetrating a cell one may question whether he is using the term *filament* as synonymous with *haustorium*, or whether he is describing intracellular filaments distinct from the haustoria whose action he described in the first article upon *Uromyces Caladii* (1929). Figure 1 printed in his first article indicates that he distinguishes between the two structures since he figures an epidermal cell which contains both a coiled structure labelled haustorium and an elongated hypha labelled filament. The legend of figure 3 reads, "Cellule traversé par un filament de rouille, Le filament a émis un suçoir." However, in the second article he labels as haustorium both a detached branching coil within the cell, and a long hypha which apparently enters from the adjoining cell. The method of penetration of these filaments, whether they are intracellular hyphae or haustoria, is, according to these figures, most unusual. There is no constriction shown at the point of entrance and the cell wall of the host is drawn without break across the full width of the hypha. This may indicate that the point of penetration is at a different plane than that of the figure. Such a condition is figured by Evans (1933) for *Urocystis cepulae* where intracellular hyphae are passing out into intercellular spaces but Evans has also figured hyphae in the penetration plane where there is a well marked aperture in the host cell wall. The constriction of hyphae as they penetrate a wall is found even in the case of such facultative parasites as *Botrytis*, *Rhizopus*, or *Pythium*.—witness the figures of Hawkins and Harvey (1919) for *Pythium debaryanum*. Since it is characteristic of the majority of rust haustoria that penetration is effected by a papilla which leaves no appreciable opening in the host cell wall and which remains as a narrow neck at the base of the haustorium, the manner of penetration of such unconstricted hyphae as Dufrenoy shows needs explanation or comment.

In figure 1 in his second article Dufrenoy figures a haustorium with a narrow neck such as I have found most typical for rust haustoria and such as I have figured for *Uromyces Caladii* in figure 56 (1927) and more strikingly in figures 2 and 6 of the present article. If *Uromyces Caladii* has both intracellular hyphae, and haustoria with differing penetration habits it would seem reasonable to expect that they might affect the host cell differently. Yet Dufrenoy treats them as the same in reaction and, as I have already noted, in his second article calls them both haustoria.

I have never found intracellular hyphae in the case of *Uromyces Caladii*. The only intracellular structures I have found are coiled and lobed haustoria which show a slender basal stalk or neck if the section is cut at the

plane of their penetration. I am convinced that these hyphae which Dufrenoy figures crossing walls of the host cells without constriction are not intracellular but are intercellular hyphae which lie at a different level from that of the cells figured, though it is hard to understand how Dufrenoy could have made such an error of observation.

Uromyces Caladii infects both epidermis and chlorenchyma of *Arisaema* leaves with a vigorous growth of intercellular hyphae and of haustoria. Intercellular hyphae make an especially thick felt in the space between lower epidermis and spongy parenchyma. Here are the anlagen of spermogonia and aecidia, and later, of teleutosori. In cross sections of leaves the intercellular spaces next the lower epidermis are often so enlarged as to seem like cells filled with hyphae. Only the observation of the cell angles, the presence or absence of the primordial utricle, and of plastids, enables one to distinguish between cell cavity and intercellular space. See figure 3, a detail from a cross section of a leaf which bore erumpent spermogonia. Here a mass of hyphae lies in an enlarged intercellular space between lower epidermis and spongy parenchyma.

Dufrenoy, however, made his observations upon tangential views of cells obtained by stripping shreds of epidermis from a leaf. Any teacher who has used strips of onion epidermis for the demonstration of protoplast structure knows the difficulties occasioned by the third dimension in tangential views of epidermis to which a layer of parenchyma often clings.

Since seeing Dufrenoy's figures and descriptions I have made studies of fresh epidermal strips in addition to those of cross sections of embedded material. I mounted the fresh material, as Dufrenoy did, in 8% sugar solution with 10% neutral red for stain. Dufrenoy also used osmic-chromic acid fixation and fuchsin stain but apparently upon similar epidermal strips and not upon embedded material.

I have shown in figure 1 a tangential view of the under epidermis of a leaf which was mounted with the outer side up. A haustorium is seen in the central cell; the curve of its narrowed base indicates that its penetration point is in a lower plane. It probably arises from a branch of the intercellular hypha which shows through the adjoining cell from below and disappears under the cell wall. Another hyphal branch may be seen, through the cell, ending beneath the stoma. The three hyphae whose tips lie apparently within, but in reality below, epidermal cells are similar to those which Dufrenoy calls filaments in his first article and haustoria in his second. The short branch beneath the lower, lefthand cell is quite similar to the branch within a cell which Dufrenoy labels haustorium in figure 3 of his second article. It is apparent that the cell walls and the cytoplasm which lie across or around the hyphae in my figure lie in an upper

plane and have no connection with the hyphae. There was no evidence of plasmolysis in any of these cells. The 8% sugar solution was isotonic for infected as well as uninfected cells instead of for the latter only as Dufrenoy states. Figure 2 shows an epidermal cell drawn from the same mount as that of figure 3, after it had stood for an hour. Evaporation had concentrated the sugar solution and all the cells were plasmolyzed but in this state there was, perhaps, even more conclusive evidence for invagination of the cytoplasm by the haustorium.

Figure 6 shows another epidermal cell which contains a haustorium. The characteristic slender neck of the haustorium may be noted. The haustorium tip lies near the centrally located nucleus. A bundle of raphides lies in the large vacuole. Here again there is evidence of invagination of the host cytoplasm by the haustorium. There is no greater evidence of fragmentation of the vacuolar system by a network of cytoplasmic strands than is often the case with centrally located nuclei in uninfected cells.

Figures 4 and 5 were drawn from a shred of lower epidermis to which patches of spongy parenchyma adhered. It was mounted inner side up. Figure 5 should overlie the right-hand part of figure 4. Figure 4 shows the median optical plane of the epidermal cells; figure 5, the median optical plane of the parenchyma cells. Two of the epidermal cells contain large, coiled haustoria, outgrowths, presumably, of intercellular hyphae which lay just above. Such a hypha is shown in figure 5, issuing from below a parenchyma cell and overlying the epidermal cells. The entire lack of plasmolysis in the infected cells is the more evident because of the tiny plastids which lie in the primordial utricle. These were leucoplasts and were sharply distinguishable in size and color from the chloroplasts of the parenchyma cells. This tissue was mounted in 8% sugar solution but was unstained.

Figures 7 and 8 show lower and upper planes respectively of an epidermal cell which was mounted inner side up. The intercellular hypha plainly overlies the cell; the lower branch of the hypha in figure 8 is similar to the one labelled haustorium in figure 3 in Dufrenoy's second article (1929). Figure 9 gives another view of the inner side of epidermal tissue drawn at the plane of the intercellular hyphae. The cytoplasm around the tip of the hypha might indicate plasmolysis if one considered the hypha to lie in the plane of the cell. Figure 10 shows a group of epidermal cells drawn in the plane of the upper outer surface. Intercellular hyphae from a lower plane show through the cells. The hypha is clearly below the nucleus of the lower cell; the tip is also below the cytoplasm which seems to surround it.

I am not prepared to check Dufrenoy's observations upon other rusts

and other fungi. My own studies of *Puccinia Sorghi* are conclusive, I believe, for invagination by haustoria but for this rust Dufrenoy gives only one figure which shows haustoria. This is a tangential view of corn cells showing a late stage of the rust with heavily encased haustoria and with inconclusive evidence, in my opinion, for either plasmolysis or invagination. The other fungi upon which Dufrenoy reports I have not studied. However, I feel that the discrepancies which I have noted in the case of *Uromyces Caladii*, the fungus of his major studies, should throw doubt upon Dufrenoy's general conclusions as to host-parasite relations.

I believe that *Uromyces Caladii*, like the other rust fungi, is a highly adapted parasite in that it neither penetrates nor plasmolyses the host cytoplasm and by its invagination makes little appreciable alteration in the physical condition of the cytoplasm.

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Explanation of plates

The figures were drawn with the aid of a camera lucida. A Spencer apochromatic objective 4 mm. and ocular 10 \times were used, with approximate magnification $\times 650$.

The material for figure 3 was fixed in Flemming's medium solution, cut 7.5 microns thick, and stained in Flemming's triple stain. The other figures were drawn from strips of epidermis mounted in 8% sugar solution. Figures 1, 2, 6, 7-10 were stained in 10% neutral red; figures 4 and 5 were unstained.

Plates 6-8

Uromyces Caladii in leaves of *Arisaema triphyllum*.

Fig. 1. Tangential view of cells of the lower epidermis which was mounted with the outer side uppermost

Fig. 2. An epidermal cell from the same mount as that of figure 1 after evaporation had so condensed the mounting fluid as to cause plasmolysis.

Fig. 3. A view from a cross section of a leaf showing intercellular hyphae in a space between the lower epidermis and the spongy parenchyma.

Fig. 4. Tangential view of cells of the lower epidermis which was mounted inner side uppermost.

Fig. 5. A higher plane of the right hand area of figure 4 showing the spongy parenchyma and a hypha which lies in the space between parenchyma and epidermis.

Fig. 6. Tangential view of an epidermal cell in the plane of the slender neck of a haustorium.

Fig. 7. Tangential view of a cell of lower epidermis which was mounted inner side uppermost. An intercellular hypha lying above the cell shows faintly.

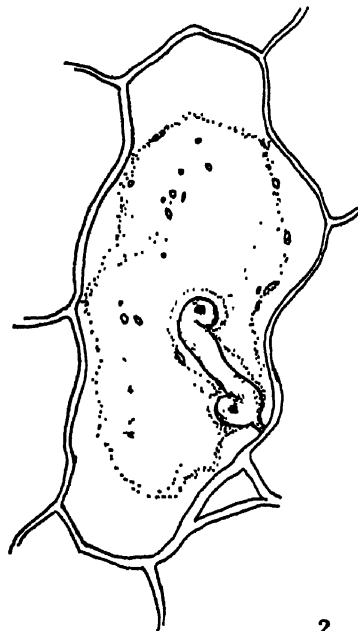
Fig. 8. A view of the same cell that is shown in figure 7 in the higher plane of the intercellular hypha.

Fig. 9. Tangential view of epidermal cells which were mounted inner side uppermost showing an overlying intercellular hypha.

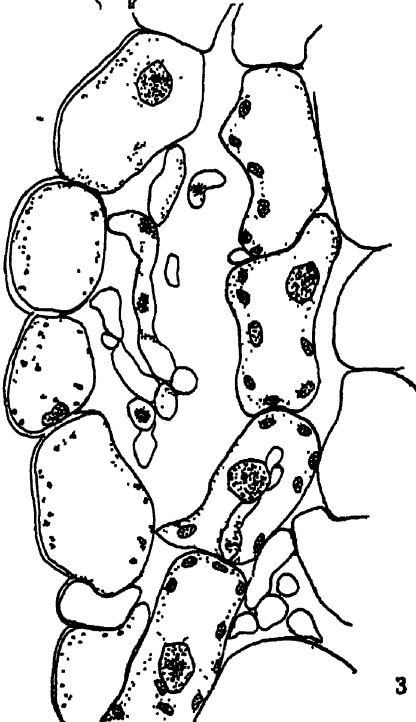
Fig. 10. Tangential view of cells of lower epidermis which was mounted outer side uppermost. An intercellular hypha which lies at a lower level shows through the cells.



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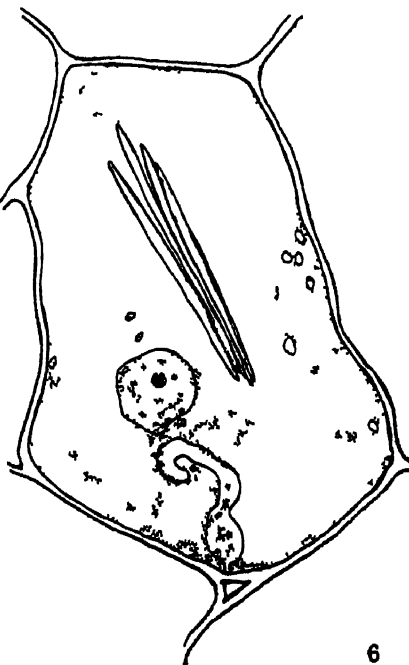
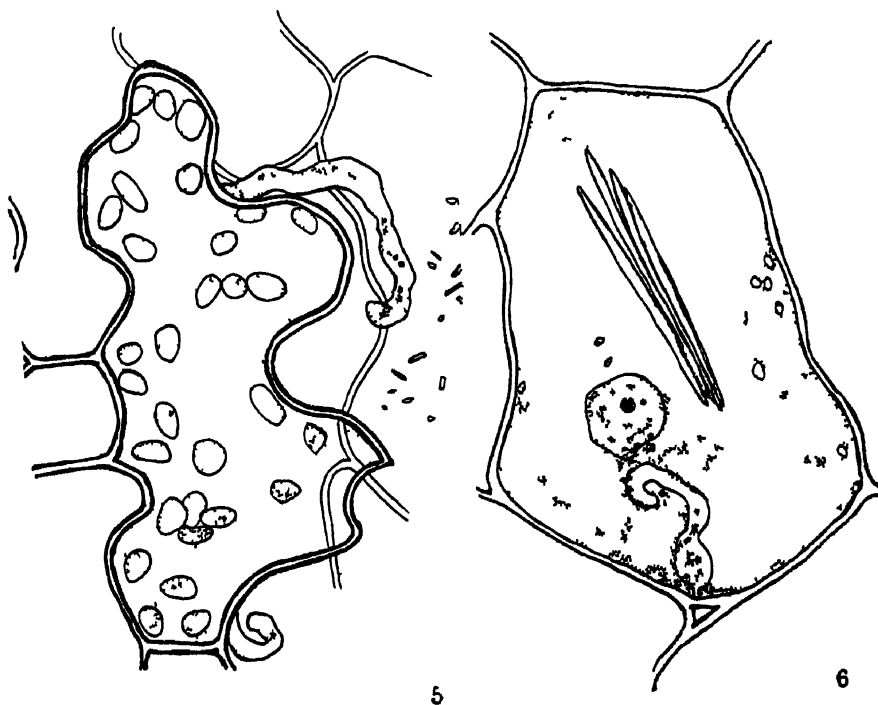
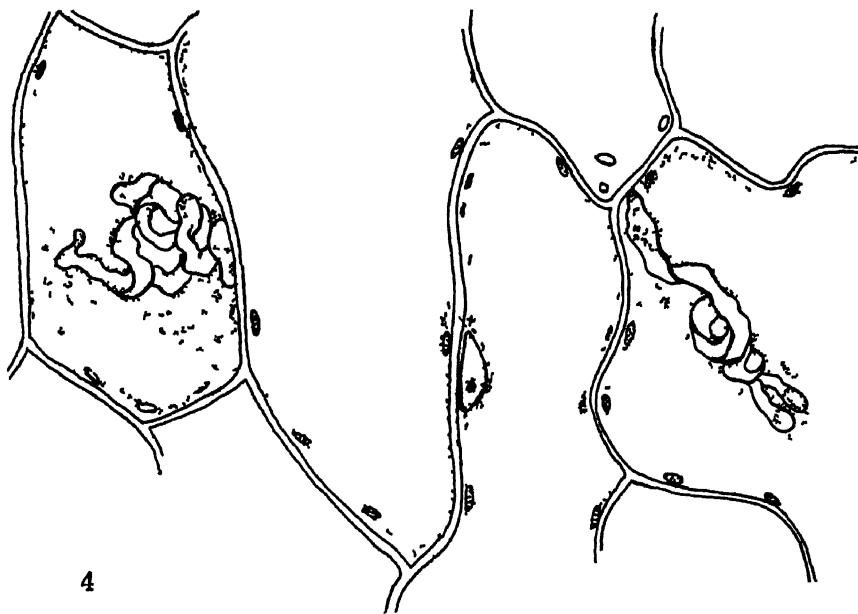


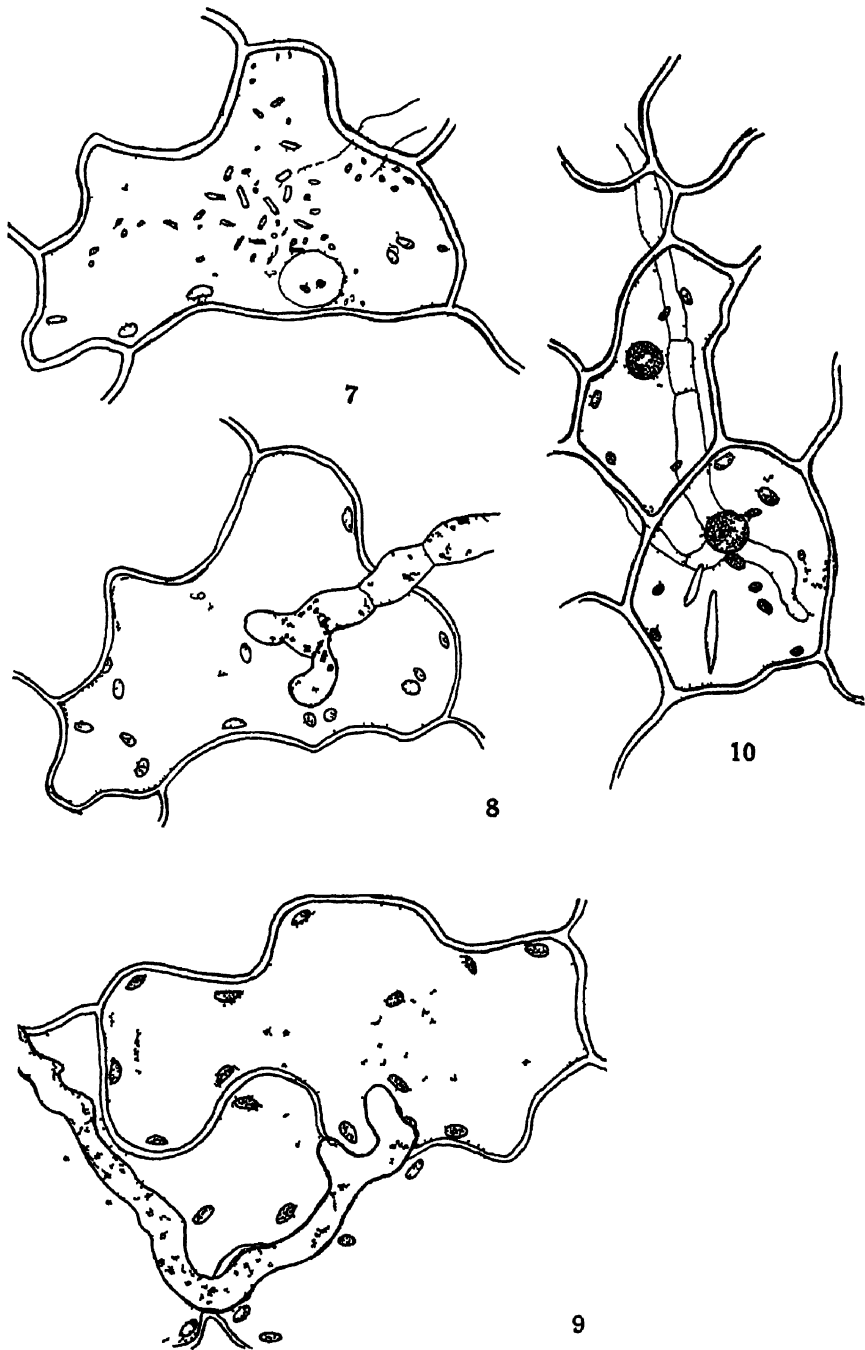
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RICE UROMYCES CALADII





RICE UROMYCES CALADII

INDEX TO AMERICAN BOTANICAL LITERATURE 1930-1933

The aim of this Index is to include all current botanical literature written by Americans, published in America, or based upon American material; the word America being used in the broadest sense.

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value of R and the former being a common temperature-oxygen combination in nature and in greenhouses.

As was to be expected, none of the cultures showed respiration water as very important when compared with water derived from the surroundings, but nevertheless 8 per cent is quite considerable. On the basis of my assumptions and computations, it appears that high growth efficiency with respect to carbon loss (G/C) naturally goes with small importance of respiration water, and conversely. The relatively great importance of respiration water shown for the combinations 30° with 20 per cent, and 30° with 90 per cent occur for a temperature that is surely supra-optimal for general health of these seedlings, with relatively rapid carbon dioxide production (C), slow growth (G) and very low growth efficiency (R); perhaps all of the seedlings were somewhat unhealthy at 30° .

Of course this little discussion of the water relations of these very young seedlings is not to be considered as well grounded on quantitative observation, but respiration water is surely worthy of more theoretical and experimental attention than it has generally thus far received in connection with the physiology of the plant organism as a whole, and it appears that its quantitative importance may vary widely according to such environmental influences as maintained temperature and oxygen pressure. Direct measurement of rates of production of respiration water appears to be difficult in the present stage of physiological technique, but measurement may be made of rates of volume increase, dry-weight change, carbon dioxide production and oxygen absorption.

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most rapid carbon dioxide production observed, with a growth-efficiency ratio (R) of 0.0172, which is relatively very low. For this combination it is estimated (see the table) that 940 mg. of water was incorporated in shoot and roots while 232.6 mg. of carbon dioxide was produced, along with 77.5 mg. of respiration water. Under these conditions the seedlings were obviously very wasteful of carbon but my method of approximation indicates that only about 91.6 per cent of the water incorporated by growth was absorbed from without, 8.2 per cent of it being derived from respiration. The combination of 30° with 20 per cent gave a similar low value of R (0.0169), showing a similar percentage importance of respiration water (8.4) but G is here only about half as great (2.2) as in the first case, C (130.2) being also much smaller.

Computation of respiration water for six representative combinations of temperature and O_2 pressure

Temperature— O_2 combination		30°, 20%	30°, 90%	25°, 75%	25°, 95%	20°, 20%	20°, 30%
Average length, 10 longest shoots, mm. (G)		2.2	4.0	8.0	12.2	7.2	9.2
Estimated mg. of H_2O incorporated by growth of 100 seedlings (235 G)		517	940	1880	2867	1692	2162
Mg. of CO_2 produced by 100 seedlings (C)		130.2	232.6	157.3	190.8	57.1	63.0
Estimated respiration water formed by 100 seedlings	Mg. ($C/3$)	43.4	77.5	52.4	63.6	19.0	21.0
	Percentage of incorporated H_2O (0.142 C/G)	8.4	8.2	2.8	2.2	1.1	1.0
Growth-efficiency index (C/G , or R)		0.0169	0.0172	0.0509	0.0639	0.1261	0.1460

For the combination of 25° with 75 per cent, only 2.8 per cent of the incorporated water appears to have been derived from respiration. This percentage is still smaller (2.2) for the combination of 25° with 95 per cent, which gave the greatest observed value of G . For the combinations 20° with 20 per cent. and 20° with 30 per cent, this percentage is very small (1.1, 1.0), the latter being the combination that gave the highest observed

the amount of water derived from respiration to the total amount incorporated in shoot and roots would thus be $C/3 \times 1/235 G$, or $C/705 G$, and this may be expressed as a percentage if multiplied by 100, giving $0.14 C/G$. But R , the index of growth efficiency with respect to carbon loss (Livingston and Mack, 1934), is G/C and so the last expression may be written $0.142 \times 1/R$. Respiration water would thus supply more of the water incorporated by growth when R is small and less when R is large. Under conditions permitting much growth per unit of carbon dioxide produced, water of respiration would therefore be less important than when much carbon dioxide is produced per unit of growth. When metabolism is, as it were, wasteful of carbon, a considerable part of the growth water may be derived from respiration, but when there is relatively little waste of carbon the quantitative importance of respiration water is small.

Liaskovskii's analysis showed his cucurbit seeds to contain much fat and protein but only a negligible proportion of carbohydrate. According to his experiments, one weight unit of H_2O was produced for each 2–5 weight units of CO_2 , the ratio of H_2O to CO_2 ranging from 1:2 to 1:4.9. In the present discussion this ratio is taken as 1:3, on the basis of the supposition that $C_6H_{10}O_5$ may fairly represent the material oxidized. If oxidation were incomplete, water production should of course be relatively less, and if material completely oxidized were such that its atomic ratio of H to C were greater than 10:6, water production should be relatively greater. According to Knüttel, Oudemans and Rauwenhoff's early experimental results lead to values of the ratio $CO_2:H_2O$ that differ according to the plant form studied and according to the developmental stage of the seedlings considered, being apparently about 1:2 for their earliest germination stage of *Fagopyrum* and of *Brassica* but only about 1:50 for their earliest stage of *Pisum*. Knüttel quoted the Dutch writers as saying that water production was first less than carbon dioxide production but that the former increased more rapidly than the latter and surpassed it as development proceeded. For a later state of *Fagopyrum* their ratio is about 1:1/2; for a later stage of *Pisum*, about 1:2/3.

For each of six representative temperature-oxygen combinations, the accompanying table shows the primary values of G and C , also the estimated total amount of water incorporated in growing shoot and roots (235 G) and the estimated amount of water produced by respiration ($C/3$). The latter is expressed in terms of milligrams for 100 seedlings in the 46-hour period and also as a percentage of the estimated total amount of water incorporated by growth. Finally, the value of the growth-efficiency index (R) is shown for the sake of comparison.

The combination of 30° with oxygen pressure of 90 per cent gave the

(*C*) and the average length of the ten longest seedlings (*G*). Results from several like tests are averaged in each instance.

The regular experimentation did not include measurements of all shoots nor did it include any root measurements at all. Some tests subsequently made by Mr. W. Luther Norem, of this Laboratory, showed that "Nittany" wheat seedlings very similar to the largest ones produced in the regular experimentation had generally a shoot diameter of about 1.2 mm., while they generally bore three roots each, about 0.6 mm. in diameter, each root being about 1.4 times as long as the shoot. For a very rough estimate, it may therefore be supposed that the increment of shoot volume per millimeter of length was generally about 2.3 cu. mm. and that the increment of root volume per millimeter of *shoot* length was generally nearly the same, about 2.4 cu. mm.; consequently the total volume increment of shoot and roots combined may be taken as about 4.7 cu. mm. per millimeter of shoot length. However, *G* is obviously larger than the average shoot length that might have resulted if all the shoots in each culture had been measured, but this inadequacy of the regular data may be approximately corrected by making the assumption that the average shoot length per culture was probably about half of the corresponding average for the ten longest shoots. To obtain an estimate of the total shoot-root volume increment per 100-seedling culture, this derived value ($G/2$) may be multiplied by 100, and $4.7 \times 100 G/2$ may be taken as the estimated total volume increment per culture; that is $235 G$, in terms of cubic millimeters.

The total shoot-root volume increment for a culture may safely be considered as very nearly equal to the volume of water incorporated in shoots and roots during the growth period of 46 hours; other materials than water may be regarded as playing a negligible part in the determination of the volume of such young tissues. Most of the water thus incorporated was doubtless derived by absorption from the surrounding nutrient solution but some portion of it must have been derived from respiration. To get a rough idea of the amount of respiration water produced by a culture that produced *C* mg. of carbon dioxide we may suppose that endosperm starch was the ultimate source of the material oxidized in respiration and that the chemical unit $C_6H_{10}O_5$ was completely oxidized in the process. From such oxidation the weight of water produced is about one-third (0.341) of the weight of carbon dioxide produced in the same time; for each 6 mols of carbon dioxide produced there should be produced also 5 mols of water, or 90 weight units of water for each 264 weight units of carbon dioxide. If these assumptions are regarded as legitimate, a culture with a total shoot-root volume increment of $235 G$ should have produced about $C/3$ mg. of water while giving off *C* mg. of carbon dioxide. The ratio of

of the plant's water supply would be "arguing in a circle," as Raber thought. It is not necessary to forget, however, that the vast majority of plant forms (bacteria, fungi, saprophytes and parasites in general) derive food from other organisms and that the material oxidized by germinating seeds or by seedlings grown in darkness was not synthesized from water and carbon dioxide by themselves; in a very true sense such organisms are saprophytic. Also, if one cares to delve at all deeply, it is desirable to consider tissues and cells, and most tissues and cells, even of ordinary green plants, derive their food from other parts of the plant body.

The availability of an extensive series of quantitative observational data on carbon dioxide production and shoot elongation in very young "Nittany" wheat seedlings led me to try to compute approximately what portion of the water used in tissue enlargement might possibly have been formed within the plantlets and what portion might have been absorbed from without. The data referred to are those reported by Mack (1930) and by Mack and Livingston (1933), who have described the experimental technique employed and the averaging computations used. They are also the ones studied by Livingston and Mack (1934). (It is to be noted that Mack and Livingston consider values for the last 36 hours of the experiment period, while Livingston and Mack consider them for the whole period, and as relative values only; but data for computing the actual ones are given by the last-mentioned writers). The standard seedlings used had just protruded their coleoptiles. They were grown for 46 hours, submerged under dilute mineral nutrient solution; consequently their external supply of water was surely adequate at all times. They were in darkness, consequently there was no photosynthesis. The amount of carbon dioxide produced by each culture, of 100 seedlings, was ascertained and the average length of the ten longest shoots of each culture at the end of the experiment period was taken as an index of shoot elongation. A specified gas mixture continually bubbled through the solution in each culture flask and a large number of different proportions of oxygen and nitrogen were tested, both without and with ethylene (0.1 per cent. by volume). Each gas mixture was tested at each of five maintained temperatures (10°, 15°, 20°, 25°, 30°) and oxygen pressures of 0.6, 6.3, 9.8, 16, 20, 30, 50 and 75 per cent, by volume, were employed with ethylene, while the same oxygen pressures and also those of 90, 95 and 98.3 per cent were tested without ethylene. The present paper is concerned only with the data for the entire experiment period of 46 hours. The accompanying table presents the data for six different combinations of temperature and oxygen pressure, without ethylene, selected as representative of the whole range. For each combination the observational values are the weight of carbon dioxide produced

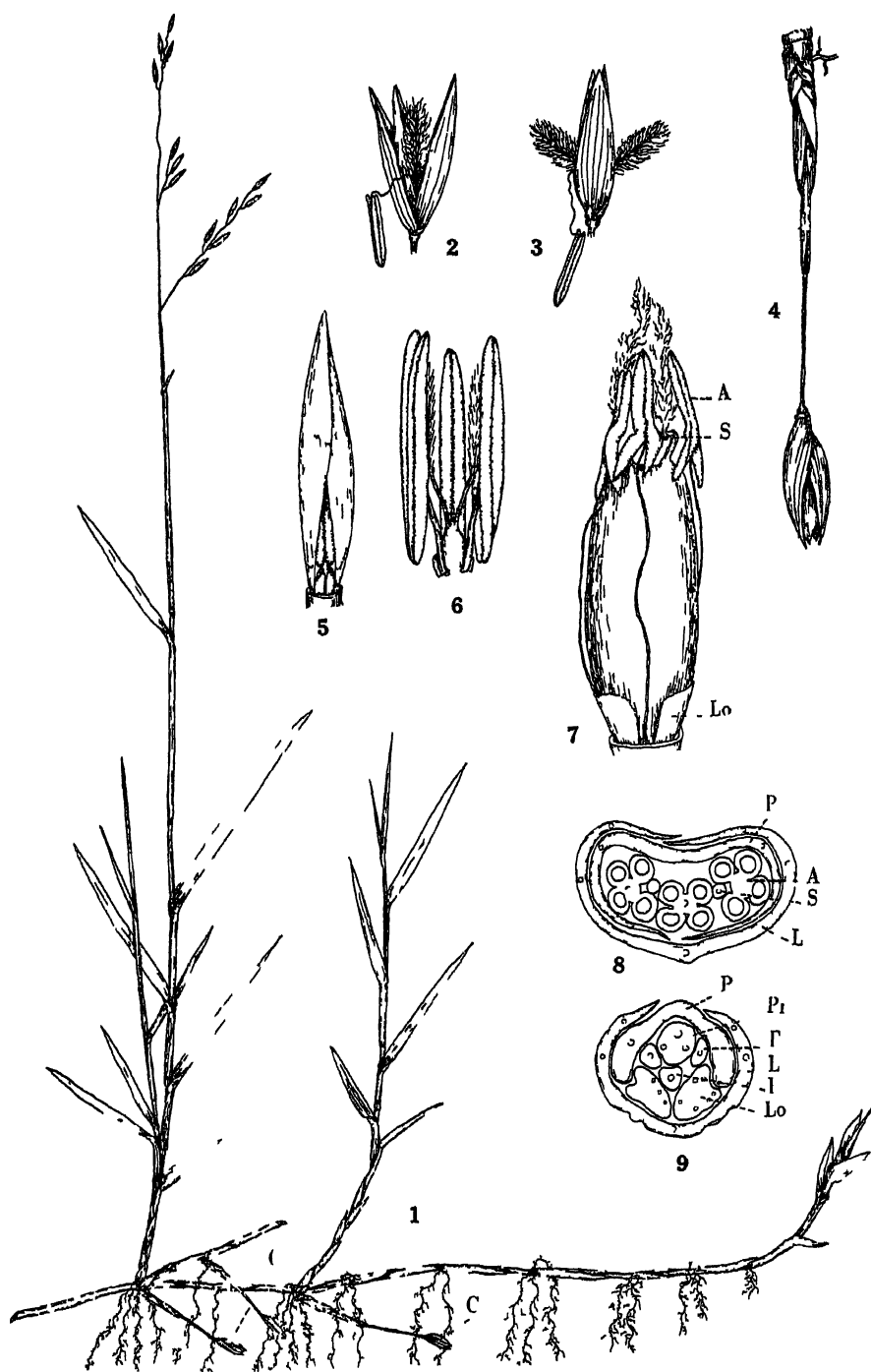
Possible importance of respiration water to young wheat seedlings¹

BURTON E. LIVINGSTON

That the respiratory process produces water as well as carbon dioxide has long been known, but the possible importance of water so derived has received but little attention. Although the amount of water metabolically formed in an experiment period is doubtless usually small when compared with the amount of water absorbed from the environment in the same period, nevertheless, respiration water is highly important in physiological theory and it surely plays a really considerable rôle in the water supply of some organisms under some sets of environmental conditions.

This topic interested de Saussure, who dealt with it in 1804. Liaskovskii's studies on seed germination, reported in 1874, are usually mentioned in recent writing on metabolic water. Liaskovskii was acquainted with the earlier studies of Oudemans and Rauwenhoff, but only through Knüttel's apparently rather complete abstract (Knüttel 1859-60). I have not seen the original Dutch paper (Oudemans and Rauwenhoff 1858), but Knüttel's German article reports that these authors made extensive experiments (about 1857) on the chemical changes that accompany seed germination, concluding, among other interesting things, that water was actually produced, as well as carbon dioxide. It appears that Babcock's discussion of this subject published in 1912, is the most thorough and the most recent, although I may have missed some later references. In 1924 Kostychev wrote, in his monograph on plant respiration (p. 11): "Die Wasserbildung bei der Pflanzenatmung ist leider unzureichend untersucht worden. Dies ist um so mehr zu bedauern, als namentlich die Ausgiebigkeit der Wasserbildung in manchen Fällen als Kennzeichen einer totalen Verbrennung des Atmungsmaterials dienen kann." He then referred to Liaskovskii's studies, pointing out that hydrolytic phenomena complicate the interpretation of such experiments, and his short paragraph ends with the words, "trotzdem ist es dringend notwendig, die so wichtige Lücke auszufüllen." Nothing was added in 1926, when the same author's text (p. 460) on chemical plant physiology appeared, nor in Lyon's translation of it (p. 380), published in 1931, excepting that Lyon cited Bonnier's (1893) researches to emphasize the importance of hydrolysis in seed germination. In Raber's recent elementary text book (1933, p. 294) the reports of Liaskovskii and Babcock are mentioned but the author seemed to think that metabolic water in plants is scarcely worth considering at all, because its hydrogen and oxygen must necessarily represent water previously reduced in carbohydrate formation; to regard the production of respiration water as a part

¹ Botanical contribution from The Johns Hopkins University, no. 125.



Figs. 1-9. *Amphicarpon floridanum*.

Fig. 1. Habit.

Figs. 2, 3. Aerial spikelets at anthesis.

Fig. 4. Cleistogamous, underground spikelet.

Fig. 5. Dorsal view of aerial floret with lemma removed.

Fig. 6. Ventral view of aerial flower.

Fig. 7. Dorsal view of cleistogamous flower.

Figs. 8, 9. Sections through aerial floret at different levels.

C, cleistogamous spikelet; A, anther; S, style; Lo, lodicule; P, palea; L, lemma
Pi, pistil; F, filament of stamen.

regularly produce a considerable number of well developed fruits, although other grasses usually produce fruits less freely under these conditions than in their normal habitats. This suggests some deficiency in the development of the pollen tube, the more humid atmosphere of the greenhouse probably being sufficient to insure success. It should be noted also that spikelets containing mature fruits readily disarticulate from their pedicels, and, consequently, data obtained from herbarium specimens will ordinarily not indicate a true state of affairs.

The subterranean spikelet is a little larger than the aerial one at the time of flowering, and the parts of the floret are much modified (fig. 7). The ovary is large, but the stigmas are small, distorted, and rudimentary. The anthers are small and short and almost sagittate in form. The anthers are closely applied to the stigmas and the whole mass is tightly wedged into the conical cavity formed at the top between the lemma and palea. The lodicules are small and apparently make no progress at all toward opening the floret. Consequently close pollination necessarily occurs.

The fruit formed underground is much larger than that of the aerial panicle. All attempts to germinate the seeds of either kind have resulted in failure, although dissection leaves no doubt of their viability, and seedlings developing from the underground seeds are frequently found.

Discussion. This plant gives unusually good evidence as to the trend toward cleistogamy in certain types of grasses. It is not a very long step from the basal cleistogamous shoots of *Danthonia*, *Sporobolus*, etc., to the specialized shoots of this genus. These modified shoots are probably the reduced remnants of axillary branches which were once terminated by panicles. In the ancestors of *Amphicarpon* these branches may have been of the nature of the basal branches of the autumnal phase of many species of *Panicum*.

The aerial floret of *Amphicarpon* also suggests a possible step in the evolution of cleistogamy. The inrolling of the edges of the stiff, indurated palea makes it so difficult for the anthers to emerge that two of them usually remain included. A slight additional induration at the base of the lemma and palea would be sufficient to prevent the hinge-like action necessary for their separation, and this condition seems to have been reached in the cleistogamous spikelets growing underground. The subsequent degeneracy of the lodicules and decrease in the size of the anthers and stigmas would be expected, because, although we have no entirely satisfactory mechanical explanation of it, the correlation between cleistogamy and the degeneracy of lodicules, anthers, and stigmas, is too consistent to be attributed wholly to coincidence.

soil north and east of Lake Okeechobee, and even in the streets of Okeechobee.

Large quantities of material have been examined in the field, and herbarium specimens and fixed material have also been studied in the laboratory. Living plants have been kept for several years in the greenhouse, where they usually flower from October to January. The plant seems intolerant of acidity in the soil and grows best in the presence of a definite alkalinity (pH 8.2 to 8.6).

Description.—The culms arise from an extensive system of branching rhizomes. Most of them are sterile, and there is a great variation from place to place in the abundance of flowering material. I have never found subterranean spikelets on plants that did not have also aerial inflorescences, and in many instances only the latter are present. The underground spikelets are frequently overlooked by the collector unless he is unusually careful or knows the peculiarity of the plant with which he is dealing.

The aerial inflorescence is a simple panicle bearing from three or four to 15 or 20 spikelets (fig. 1). The shoots bearing the underground spikelets arise singly or in clusters from near the base of the aerial culm or from elsewhere on the rhizome. Each of these shoots (fig. 4) bears a number of scale leaves and is terminated by a single spikelet. Other details can be seen in the figures.

The spikelets are much like those of the other *Panicaceae*. The lower floret is represented by only an empty lemma. The first glume is minute in the aerial spikelet and obsolete in the underground spikelet (figs. 2-4). Other differences between the two are correlated with the conditions under which they develop and with their behavior at the time of flowering.

The aerial spikelet and flower have in general the structure common to grasses that are pollinized by the wind (figs. 2, 3, 5, 6). The lodicules, which are large and well developed, function very effectively, pushing the lemma and palea wide apart, and the dorsal anther emerges on a long filament and opens and releases its pollen. The two lateral stamens, however, usually remain enclosed by the palea whose inrolled edges will not permit them to be exerted (figs. 5, 8). These imprisoned anthers release their pollen before the emergence of the stigmas, so that close pollination might normally occur if the floret did not open at all. Any stigma that fails to be effectively pollinized in this way has, of course, other chances when the dorsal anthers of flowers in other spikelets emerge. In some cases two or all three of the stamens of a flower may emerge as in typical grasses.

The regular abortion of most of the potential fruits of the aerial spikelets is almost certainly due to some factor operating at the time of flowering, because development stops at this point. My plants in the greenhouse

Flowering and seed production in *Amphicarpon floridanum*¹

PAUL WEATHERWAX

(WITH TEXT FIGURES 1-9)

Cleistogamy has arisen in the Gramineae in several different ways, and probably the most highly specialized of these occurs in three species which are known to bear fertile spikelets on underground shoots. Pursh described the first of these as *Milium amphicarpum* in 1814. This was afterwards placed in a new genus and has since been known in the manuals as *Amphicarpon*² *Purshii* Kunth or *A. amphicarpon* (Pursh) Nash. A second species, *A. floridanum*, was later described by Chapman. In a recent monograph³ on *Paspalum* Mrs. Chase describes a West Indian species, *P. amphicarpum* Ekman, which has a similar underground inflorescence.

The taxonomic descriptions state that ordinarily only the underground spikelets of *Amphicarpon* are fertile. Bentham and Hooker⁴ and Baillon⁵ even go so far as to consider the plant monoecious, the aerial spikelets being described as staminate and those underground as pistillate. The impossibility of pollination under these conditions apparently did not occur to them. Chapman⁶ says that the aerial flowers are rarely fruitful. Mrs. Chase⁷ reports that she found a few fruits in aerial inflorescences of *A. amphicarpon* which were observed in the field. In *Paspalum amphicarpum* both the aerial and the subterranean inflorescences produce fruits.

This seems to comprise all that has been published on the flowering and fruiting of these species, and it is obvious that little of this information has come from observations made in the field. I have had opportunity in the past few years to examine *Amphicarpon floridanum* in most stages of its life history, and some interesting facts may now be added to what has been known.

Material. One interested in this species need never be limited by lack of material, for it is found in great abundance in many parts of Florida. It is common along roads and railroads, in the edges of fields, and in open pasture land in all the central part of the state where the soil is suitable, and thousands of acres of the prairie region north of Lake Okkechobee are covered with it. I have found the best flowering material along road grades across the marshes just west of Groveland and on the calcareous sandy

¹ Publication No. 63 of the Waterman Institute, Indiana University.

² Also spelled *Amphicarpum*.

³ Contrib. U. S. Nat. Herb. **28**: 1-310. 1929.

⁴ Genera Plantarum. **3**: 1099. 1883.

⁵ Histoire des Plantes. **12**: 304. 1894.

⁶ Flora of the Southern U. S. 1884.

⁷ Bot. Gaz. **45**: 135-136. 1908.

The translocation of the $\cdot 2$ half to the $11 \cdot 12^\circ$ chromosome was sufficiently subterminal to permit the functioning of the terminal attachment point at the $\cdot 11$ end. The $2 \cdot 11 \cdot 12^\circ$ chromosome therefore has three attachment points.

The rod-shaped $\cdot 1$ fragment shows attachment only at that end which is the $\cdot 1$ end of the former $1 \cdot 2$ chromosome. The other end of this fragment has the spindle fiber of the broken $1 \cdot 2$ chromosome, the break having occurred apparently at the point where the spindle fiber is inserted in the $1 \cdot 2$ chromosome.

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$$1 \cdot 1 \text{ --- } 1 \cdot 2 \text{ --- } 2 \cdot 11 \cdot 12^\circ$$

$$\qquad \qquad \qquad \begin{matrix} < & > \\ & 11 \cdot 12^\circ \end{matrix}$$

From these compensations it is evident that the $\cdot 1$ fragment supplies the chromosomal material which is lacking in the $2 \cdot 2$ chromosome to furnish a complete $1 \cdot 2$ chromosome; and that the translocated $\cdot 2$ fragment similarly supplies what is lacking in the $1 \cdot 1$ chromosome. Together the $1 \cdot 1$ and the $2 \cdot 2$ chromosomes form the equivalent of a $1 \cdot 2$ chromosome plus additional material. If, when taken together, these two secondary chromosomes left a deficiency for the $1 \cdot 2$ chromosome, only one and not both of the two compensating types given above would be expected. In consequence a $1 \cdot 1$ plus a $2 \cdot 2$ chromosome might be equivalent to more than two $1 \cdot 2$ chromosomes but could not be equal to less.

The exact mechanism involved in the formation of a secondary chromosome is not understood and need not concern us in the present discussion. It is sufficient to say that the process in some way assures a locus for the insertion of a spindle fiber in the middle of the double-ended chromosome that results.

In view of the evidence available, we believe that the breakage resulting in the translocation under discussion was at essentially the same point where the breakage which resulted in the $1 \cdot 1$ and the $2 \cdot 2$ secondary chromosomes took place; and that this point corresponds to the locus of the spindle fiber insertion which separates the $1 \cdot 2$ chromosome into two unequal arms.

SUMMARY

As a result of radiation treatment there was produced a gamete in which the $1 \cdot 2$ chromosome was broken in the mid-region. The $\cdot 2$ half was translocated to the $\cdot 11$ end of the $11 \cdot 12^\circ$ chromosome, forming the $2 \cdot 11 \cdot 12^\circ$ chromosome. The $\cdot 1$ half remained free as a rod-shaped chromosome.

Cytologically three types of normal-appearing plants were found in the offspring: those like normal diploids from which the translocation arose, those which were heterozygous and those which were homozygous for the translocation (PT6). The latter has the formula: $(\cdot 1)_2 + (2 \cdot 11 \cdot 12^\circ)_2$.

Cytologically two types which had a single extra dose of $\cdot 2$ material were distinguished: $(1 \cdot 2)_2 + 2 \cdot 11 \cdot 12^\circ$ and $12 \cdot 11$ and $1 + 1 \cdot 2 + (2 \cdot 11 \cdot 12^\circ)_2$. They resembled the $2n + 2 \cdot 2$ secondary type called "Sugarloaf."

A true-breeding Sugarloaf type (PT5) was obtained: $(1 \cdot 2)_2 + (2 \cdot 11 \cdot 12^\circ)_2$. It cannot be separated from the $2n + 2 \cdot 2$ secondary by external appearance but it is easily distinguished both cytologically and genetically.

chromosome by the spindle fiber which is inserted here. This end was formerly the mid-region of the 1·2 chromosome which was broken.

Familiarity with the 1·2 or L chromosome has shown that the point at which the spindle fiber is inserted is slightly sub-median. The two arms of this V-shaped chromosome therefore are somewhat unequal. In figure 14 this 1·2 chromosome is shown drawn from sectioned material that had been fixed in a modified Carnoy and stained with Heidenhain's hematoxylin. Cytological evidence favors the 1 arm as the longer in the 1·2 chromosome.

Continued use of the 1·1 and 2·2 secondaries as testers in crosses with prime types indicates that the 1·1 chromosome is the longer. Thus the 1·1 chromosome in figure 16 appears to be longer than the 2·2 chromosomes in figures 9, 10 and 15. Definite cytological proof that this is the case requires the presence of both the 1·1 and 2·2 chromosomes in the same pollen-mother-cell. By proper breeding procedure, we are attempting to obtain such a plant with the two secondaries together. If secondary chromosomes are formed by the doubling of a single arm in all cases, the apparent size differences found in these secondaries is indirect evidence that the 1 arm in the 1·2 chromosome is the longer.

In all the five figures in which both the free and the translocated fragments are shown together in the same pollen-mother-cell, the 1 fragment appears to be the longer. The size difference may be slightly exaggerated on account of the fact that the 1 fragment has an attachment at one end only.

In *Datura*, as in other forms, the accumulating evidence seems to indicate that at least the majority of chromosomal deficiencies are lethal when homozygous. Prime type 6, which is homozygous for both the free and the translocated fragments, is viable. From the assumed effects of deficiencies, it would follow, therefore, that no part of the 1·2 chromosome was lost as a consequence of the translocation under consideration. Apparently the break occurred at the point where the spindle fiber is inserted, the spindle fiber going with the free fragment.

The 1 fragment can compensate with the 2·2 chromosome to furnish the equivalent of the 1 2 chromosome which is lacking entirely in the pure-breeding type $\left(\begin{smallmatrix} 1 \\ 2 \ 2 \end{smallmatrix} \right)_2$ shown in figure 15. The 2 fragment can compensate with the 1 1 chromosome to furnish the equivalent of a 1 2 chromosome in the compensating type $\frac{1 \cdot 1}{2 \ 11 \ 12^\circ}$ as shown in figure 16.

The diagram of the latter may be represented as follows:

1·2 chromosome at the ·1 end. Additional evidence regarding the identity of the free fragment follows.

The primary $2n+1\cdot2$ type heterozygous for PT6 shows a chain of five chromosomes in figure 12. The free fragment is attached to the chain between the two 1·2 chromosomes at a point known to be the ·1 end because of the orientation of that 1·2 chromosome which lies next to the $2\cdot11\cdot12^\circ$ chromosome. The free fragment is easily recognized by the attenuated appearance of its unattached end. The $11\cdot12^\circ$ chromosome is recognized by its size and by the hump at the ·12° end; the $2\cdot11\cdot12^\circ$ chromosome by its size, appearance and position in the chain. The L or 1·2 chromosomes must have the orientation given in the following diagram:

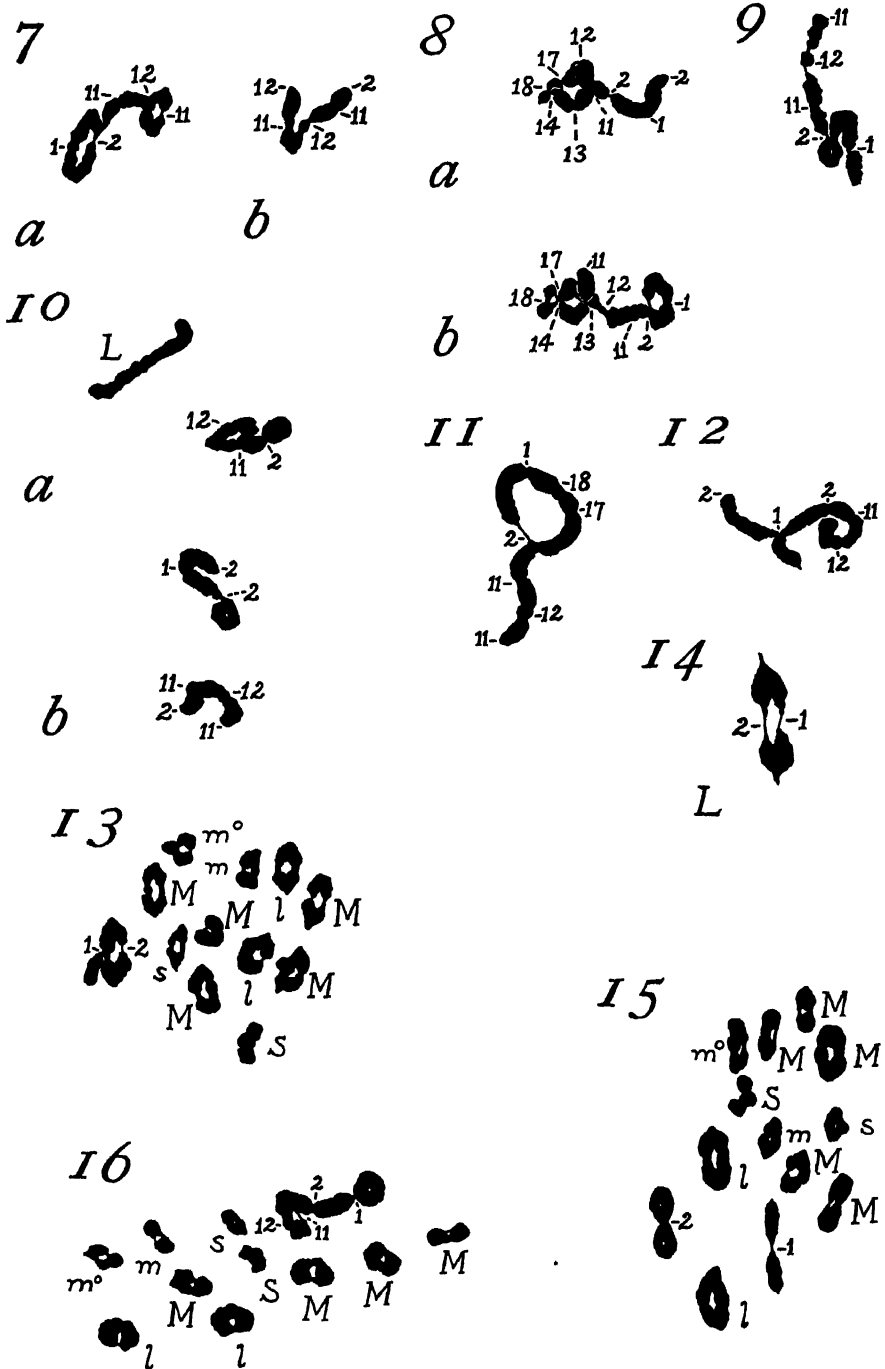
$$\begin{array}{ccc} & 1\cdot2 & \\ \cdot1 & - < > & 2\cdot11\cdot12^\circ \\ & 1\cdot2 & < > \\ & & 11\cdot12^\circ \end{array}$$

The fragment which is attached to the $(1\cdot2)_2$ chromosomes must have a ·1 end on account of its position in the configuration.

Morphological evidence that this free fragment is the ·1 half of the 1·2 chromosome was demonstrated in a plant which had this fragment as excess material. In appearance the plant resembled somewhat the $2n+1\cdot1$ secondary type but it departed less widely from normal. Its chromosomes are shown in figure 13.

Datura chromosomes have median or sub-median spindle fiber insertion regions, with the result that they are V-shaped at metaphase I. Terminal attachment points are at each end of these chromosomes. When the 1·2 chromosome was broken, the ·2 fragment became united by its inner surface at the point of the break to the $11\cdot12^\circ$ chromosome. The attachment point at the ·2 end therefore is free. Moreover the union of the ·2 fragment to the ·11 end was slightly subterminal so that the ·11 attachment point also is free. Therefore the $2\cdot11\cdot12^\circ$ chromosome has three attachment points instead of the usual two. The functioning of all three of these points is shown in figures 3a and 6a b when two $2\cdot11\cdot12^\circ$ chromosomes are present and in figures 1b, 4a-b, 8a, 10a and 16 when only one is present. Attachment often is lacking between the two ·11 ends; this leaves only the ·2 and ·12° ends functioning as shown in figures 3b, 5 and 6c d when two $2\cdot11\cdot12^\circ$ chromosomes are present and in figures 7a, 8b, 9, 11 and 12 when only one is present.

The $(\cdot1)_2$ chromosomes form a rod-shaped pair united by the attachment points at the ·1 end of each. The opposite end of each is distinguishable by being somewhat ragged and drawn out as though the end of the coiled chromonema were loosened and pulled away from the body of the



Figs. 7-16

The fragment translocated to the $11 \cdot 12^\circ$ chromosome is attached to the circle of four between the two L chromosomes $1 \cdot 2$ and $2 \cdot 17$, thus showing that it possesses a $\cdot 2$ end.

Several independent lines of argument, therefore, have shown that it is the $\cdot 2$ fragment which was translocated to the $11 \cdot 12^\circ$ chromosome. From inspection of figure 1 (which is an example of heterozygous PT6), it will be seen that the free fragment must be $\cdot 1$ since it is attached to the

Explanation of figures 7-15

Fig. 7 *a* and *b*. Chromosomal configurations found in a $2n + 11 \cdot 12^\circ$ plant heterozygous for PT5. In (*a*) the $2 \cdot 11 \cdot 12^\circ$ chromosome connects the two closed bivalents $(1 \cdot 2)_2$ and $(11 \cdot 12^\circ)_2$; the ten bivalents have been omitted. In (*b*) a chain of three chromosomes $(11 \cdot 12^\circ)_2 - ^\circ 12 \cdot 11 \cdot 2$ is shown, the eleven bivalents having been omitted.

Fig. 8 *a* and *b*. Configurations of eight chromosomes found in a plant heterozygous for PT5 and PT11. In (*a*) the $(1 \cdot 2)_2$ bivalent is open; in (*b*) the circle is broken at the $\cdot 11$ attachment points. The formula of this configuration is $(1 \cdot 2)_2 - 2 \cdot 11 \cdot 12^\circ - ^\circ 12 \cdot 17 - 17 \cdot 18 - 18 \cdot 14 - 14 \cdot 13 - 13 \cdot 11$.

Fig. 9. A chain of five chromosomes found in a $2n + 2 \cdot 2$ plant which is heterozygous for PT6. The $2 \cdot 2$ chromosome forms a "doughnut" between the $1 \cdot 2$ and $2 \cdot 11 \cdot 12^\circ$ chromosomes. The chain is represented as $\cdot 1 - 1 \cdot 2 - 2 \cdot 2 - 2 \cdot 11 \cdot 12^\circ - ^\circ 12 \cdot 11$.

Fig. 10 *a* and *b*. Chromosomal configurations found in $2n + 2 \cdot 2$ plants which are heterozygous for PT5. In (*a*) the $2 \cdot 2$ chromosome is attached to the $\cdot 2$ end of the $2 \cdot 11 \cdot 12^\circ$ chromosome; in (*b*) it is attached to the $(1 \cdot 2)_2$ bivalent. These broken chains have the formula $(1 \cdot 2)_2 - 2 \cdot 2 - 2 \cdot 11 \cdot 12^\circ - ^\circ 12 \cdot 11$. The ten bivalents have been omitted.

Fig. 11. Configuration of six chromosomes found in a plant heterozygous for PT2 and PT5. Formula for this configuration is $11 \cdot 12^\circ - ^\circ 12 \cdot 11 \cdot 2 - 2 \cdot 17 - 17 \cdot 18 - 18 \cdot 1 - 1 \cdot 2$.

Fig. 12. Chain of five chromosomes found in a $2n + 1 \cdot 2$ plant heterozygous for PT6. The free fragment is attached to the chain at the $\cdot 1$ ends of the $(1 \cdot 2)_2$ chromosomes. The formula is $\cdot 1 - (1 \cdot 2)_2 - 2 \cdot 11 \cdot 12^\circ - ^\circ 12 \cdot 11$.

Fig. 13. Chromosomal configuration of a $2n + 1$ plant. The $\cdot 1$ fragment is attached to the $(1 \cdot 2)_2$ bivalent.

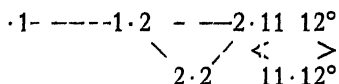
Fig. 14. The $(1 \cdot 2)_2$ bivalent drawn at a magnification of 2900 diameters to show that one arm ($\cdot 1?$) is slightly longer than the other ($\cdot 2?$).

Fig. 15. Chromosomal configuration of a pure-breeding 26-chromosome plant $\left(\frac{\cdot 1}{2 \cdot 2} \right)_2$. The $(\cdot 1)_2$ bivalent is recognized as a pair of rod shaped chromosomes; the two attached $2 \cdot 2$ chromosomes form a "dumbbell."

Fig. 16. Group of four chromosomes found in the 24-chromosome $\left(\frac{1 \cdot 1}{2 \cdot 11 \cdot 12^\circ} \right)$ compensating type. The formula is $1 \cdot 1 - 1 \cdot 2 - 2 \cdot 11 \cdot 12^\circ - ^\circ 12 \cdot 11$.

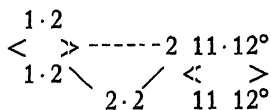
Proof will now be given that the fragments come from the 1·2 chromosome and that it is the ·2 half which was translocated to the 11·12° chromosome. It was indicated by the size of the fragments and by their attachments to the L chromosomes, when they were heterozygous, that they are parts of the 1·2 chromosome. This conclusion was confirmed by the evidence which determined the identity of the fragments.

The secondary $2n+2\cdot2$ plant heterozygous for PT6 shows a chain of five chromosomes. The 2·2 chromosome was identified by a tendency to bend back on itself to form a "doughnut," a characteristic of secondary chromosomes. This method of using a secondary chromosome to identify the ends of a modified chromosome has been described in a previous publication.¹ In figure 9, the 2·2 chromosome may be seen lying between the 1·2 or L chromosome and the X fragment which was translocated to the 11·12° chromosome. Diagrammatically it may be represented as follows:

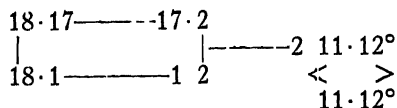


Therefore this X fragment has the ·2 end of the 1·2 chromosome and the modified chromosome is correctly written as $2 \cdot 11 \cdot 12^\circ$.

Prime type 5 may be used in the test as well as PT6. A secondary $2n+2\cdot2$ plant heterozygous for PT5 may show the 2·2 chromosome lying between a 1·2 and the X·11·12° chromosome in a chain of five. However, since there are two 1·2 chromosomes they tend to form an independent pair and the chain therefore is usually broken. This is the case in figure 10 which illustrates this cross. The same argument used for identifying the ends in a $2n+2\cdot2$ plant heterozygous for PT6 applies to a $2n+2\cdot2$ plant heterozygous for PT5, the diagram for which may be written as follows:



A cross between PT5 and PT2, the so-called "B" race which has the chromosomes 1 18(m) and 2·17(L) instead of the Line 1 chromosomes 1 2(L) and 17·18(m), results in a configuration of six chromosomes consisting of a circle of four to which an M-sized bivalent is attached. This is shown in figure 11. It can be proved to have the attachment shown in the following diagram: ‡



In the previous paragraphs are given the chromosomal constitutions of the original translocation and the different derivatives from it. How the ends have been identified may be seen from the following paragraphs.

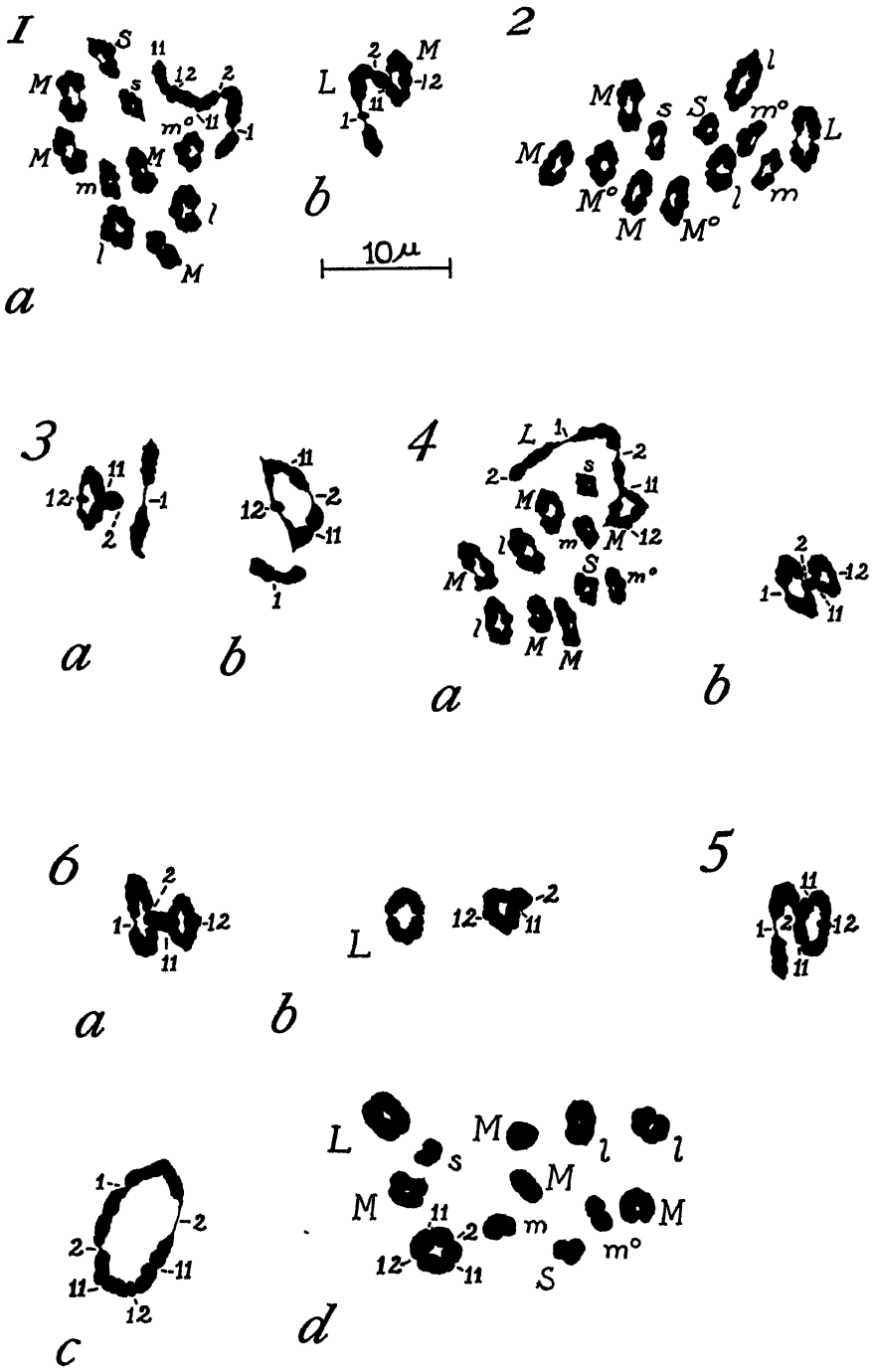
The primary $2n+11 \cdot 12^\circ$ plant heterozygous for PT5 (which it will be shown has the chromosome $2 \ 11 \cdot 12^\circ$) showed a chain of five chromosomes as illustrated in figure 7*a*. Therefore the M-sized $11 \cdot 12^\circ$ chromosome is involved in this translocation. In figure 7*a*, an M-sized bivalent and an L-sized bivalent are joined together by a chromosome slightly larger than L and somewhat bipartite. The M-sized bivalent is obviously $(11 \cdot 12^\circ)_2$; the L-sized bivalent is $(1 \ 2)_2$. The connecting chromosome must consist of an $11 \cdot 12^\circ$ chromosome to which the fragment was translocated, the point of union being indicated by a slight constriction, hence the bipartite appearance. The $\cdot 12$ end of the $11 \cdot 12^\circ$ chromosome has a terminal hump as represented by a $^\circ$ sign. Since the fragment was translocated to the end which is opposite the humped or $\cdot 12^\circ$ end, it must have been translocated to the $\cdot 11$ end. This is clearly indicated in figure 7*b*. This connecting chromosome, therefore, must be $X \cdot 11 \cdot 12^\circ$, the X representing the translocated fragment. The chain of five shown in figure 7*a* may be represented as follows:

$$\begin{array}{ccc} \begin{array}{c} 2 \ 1 \\ < \quad > \\ 2 \ 1 \end{array} & - & \text{or} \quad \begin{array}{c} 1 \ 2 \\ < \quad > \\ 1 \cdot 2 \end{array} & X \ 11 \ 12^\circ & \begin{array}{c} {}^\circ 12 \ 11 \\ < \quad > \\ {}^\circ 12 \ 11 \end{array} \end{array}$$

This conclusion that the $11 \ 12^\circ$ chromosome is the host chromosome and that the fragment was translocated to the $\cdot 11$ end was verified by crossing PT5 to PT11. The latter has the modified chromosomes $11 \cdot 13$, ${}^\circ 12 \cdot 17$, $14 \cdot 18$ instead of the Line 1 chromosomes $11 \cdot 12^\circ$, $13 \cdot 14$ and $17 \cdot 18$.¹ The $14 \cdot 18$ chromosome is recognizable because of its miniature size, the ${}^\circ 12 \cdot 17$ because of its being 1-sized with a terminal hump at the $\cdot 12^\circ$ end. The configuration of eight chromosomes induced by this cross consists of a circle of six connected with an L or $1 \cdot 2$ bivalent and may be represented as follows:

$$\begin{array}{ccccccc} 18 \cdot 17 - & - & 17 \ 12^\circ - & {}^\circ 12 \ 11 \ X & \begin{array}{c} 2 \ 1 \\ < \quad > \\ 2 \ 1 \end{array} & \text{or} & \begin{array}{c} 1 \ 2 \\ < \quad > \\ 1 \cdot 2 \end{array} \\ | & & & | & & & \\ 18 \cdot 14 - & & 14 \ 13 - & 13 \ 11 & & & \end{array}$$

This circle sometimes breaks into a chain as shown in figure 8*b*. That chromosome in the circle which connects ${}^\circ 12 \cdot 17$ with the $(1 \cdot 2)_2$ bivalent must therefore be ${}^\circ 12 \ 11 \ X$ as shown in the above diagram. The $\cdot 11$ end of the $11 \cdot 13$ chromosome connects with the original $\cdot 11$ end of the ${}^\circ 12 \cdot 11 \cdot X$ chromosome, leaving the translocated fragment as a projection from this circle as shown in figure 8*a*. It is this X fragment which connects the $(1 \cdot 2)_2$ bivalent with the circle.



Figs. 1-6

The third type of Sugarloaf is morphologically indistinguishable from the $2n+2\cdot2$ secondary type because it has two $\cdot2$ halves of the $1\cdot2$ chromosome in excess. Cytologically, however, the two are easily distinguished. The secondary $2n+2\cdot2$ has 25 chromosomes whereas this Sugarloaf type obtained from 27377(1) has 24 chromosomes. In a previous publication¹ it was listed as PT5. It is shown in figure 6 and may be represented as follows:

$$\begin{array}{ccc} \begin{array}{c} 1\cdot2 \\ < > \\ 1\cdot2 \end{array} & \text{-----} & \begin{array}{c} 2\cdot11\cdot12^\circ \\ < < > \\ 2\cdot11\cdot12^\circ \end{array} \end{array} \quad \text{or as} \quad \begin{array}{ccc} 1\cdot2 & \text{---} & 2\cdot11\cdot12^\circ \\ | & & | \\ 1\cdot2 & & 2\cdot11\cdot12^\circ \end{array}$$

Prime type 5 may be further distinguished from the secondary type through breeding behavior. Having 24 chromosomes, it is pure-breeding, whereas the secondary $2n+2\cdot2$ Sugarloaf, like all 25 chromosome types, throws both normals and its own type among its offspring.^b

^a Blakeslee, A. F., A. D. Bergner and A. G. Avery. 1933. Methods of synthesizing pure-breeding types with predicted characters in the jimson weed, *Datura stramonium* Proc. Nat. Acad. Sci. 19: 115-122.

Explanation of figures 1-6

Fig. 1 *a* and *b*. Chromosomal configuration of plants which are heterozygous for the translocation: (*a*) chain of four chromosomes formed when there is no attachment between the two $\cdot11$ ends; (*b*) "kite-like" configuration of four chromosomes formed when the $\cdot11$ ends are attached. The numerical formula for these four chromosomes is $\cdot1-1\cdot2-2\cdot11\cdot12^\circ-12\cdot11$. In (*b*) the ten bivalents have been omitted.

Fig. 2. Twelve bivalents found in normal diploids (standard Line 1).

Fig. 3 *a* and *b*. The two bivalents of homozygous PT6 which are different from bivalents found in normal diploids (standard Line 1). In (*b*) there is no attachment between the two $\cdot11$ ends. The bivalents are $(\cdot1)_2$ and $(2\cdot11\cdot12^\circ)_2$.

Fig. 4 *a* and *b*. Chromosomal configuration of a weakly Sugarloaf type which contains the $\cdot2$ half of the $1\cdot2$ chromosome as excess material. In (*a*) the $\cdot2$ ends of the two $1\cdot2$ chromosomes are not attached; in (*b*) they are. In (*b*) the ten bivalents have been omitted. The formula for this type, which is heterozygous PT5, is $(1\cdot2)_2-2\cdot11\cdot12^\circ-12\cdot11$.

Fig. 5. Chromosomal configuration of the second weakly Sugarloaf type. Its formula is $\cdot1-1\cdot2-(2\cdot11\cdot12^\circ)_2$. The ten bivalents have been omitted.

Fig. 6 *a*, *b*, *c*, and *d*. Chromosomal configurations of plants homozygous for PT5, a 24-chromosome, pure-breeding Sugarloaf type. In (*a*) the $(1\cdot2)_2$ bivalent is connected with the $(2\cdot11\cdot12^\circ)_2$ bivalent; the $\cdot11$ ends are attached. In (*b*) and (*d*) the bivalents are separate. In (*b*) the $\cdot11$ ends are attached. In (*c*) the four chromosomes have opened out to form a large circle. The ten bivalents have been omitted in (*a*) (*b*) and (*c*).

able from the $2n+2 \cdot 2$ type. Cytologically six types could be distinguished among these offspring.

There were three types among the diploids. First there were those which showed a "kite" or chain of four chromosomes like the parent. They were heterozygous PT6, which may be represented as follows:

$$\cdot 1 \text{---} 1 \cdot 2 \text{---} 2 \cdot 11 \cdot 12^\circ$$

$$\begin{array}{c} < > \\ 11 \cdot 12^\circ \end{array}$$

Another type had 12 bivalents like those of our standard Line 1 shown in figure 2. They may be represented as follows:

$$\begin{array}{c} 1 \cdot 2 \\ < > \\ 1 \cdot 2 \end{array} + \begin{array}{c} 11 \cdot 12^\circ \\ < > \\ 11 \cdot 12^\circ \end{array}$$

The third type also showed 12 bivalents but was distinguishable from the Line 1 diploids because of the appearance of two of the bivalents. Being homozygous PT6, this type may be represented as follows:

$$\cdot 1 \text{---} 1 \cdot + 2 \cdot 11 \cdot 12^\circ$$

$$\begin{array}{c} < > \\ 2 \cdot 11 \cdot 12^\circ \end{array}$$

In figure 3, the $(\cdot 1)_2$ fragments can be recognized as a pair of rod-shaped chromosomes attached to one another at one end only, in contrast to the usual pairs of V-shaped chromosomes which are attached at both ends. The $2 \cdot 11 \cdot 12^\circ$ pair is also distinguishable because the $(\cdot 2)_2$ fragments show as a projection from the closed bivalent formed by the $(11 \cdot 12^\circ)_2$ host chromosomes as shown in figure 3a. If the $\cdot 11$ ends fail to attach, the $(2 \cdot 11 \cdot 12^\circ)_2$ pair forms a large circle as shown in figure 3b.

The Sugarloaf types contain excess $\cdot 2$ chromosomal material. Cytologically two types were distinguished which were weakly Sugarloaf in appearance. The one type showed two bivalents joined by the translocated fragment as shown in figure 4. It may be represented as follows:

$$\begin{array}{c} 1 \cdot 2 \\ < > \\ 1 \cdot 2 \end{array} \text{---} 2 \cdot 11 \cdot 12^\circ$$

$$\begin{array}{c} < > \\ 11 \cdot 12^\circ \end{array}$$

The other type showed a "kite-like" configuration as shown in figure 5 and represented as follows:

$$\cdot 1 \text{---} 1 \cdot 2 \text{---} \begin{array}{c} 2 \cdot 11 \cdot 12^\circ \\ < < > \\ 2 \cdot 11 \cdot 12^\circ \end{array}$$

Both types have the $\cdot 2$ half of the $1 \cdot 2$ chromosome in excess over the normal chromosomal material.

Cytology of a translocation of the 1·2 chromosome in *Datura*

A. DOROTHY BERGNER AND A. F. BLAKESLEE

(WITH FIGURES 1-16)

Simple translocations form one of the kinds of chromosomal change brought about by radiation treatment. A chromosome is broken into two parts, one of which is translocated to a host chromosome while the other part remains as a free fragment. Among the 80 prime types,¹ which have been rendered homozygous for modified chromosomes, about 20 per cent have been classified as due to simple translocations. The first of this kind of chromosomal change found in *Datura* involved the 1 2 which is the largest (L) chromosome. A brief note concerning the translocation was published earlier.² It was mentioned in a discussion of prime types¹ where it was listed as P'T6. The present paper will give an account of the cytology of this translocation while another paper will discuss the unbalance brought about by the products of the translocation when present as extra material.

During the summer of 1928 a cytological examination was made of some plants which had been obtained following radiation treatment. Included in the group was the plant number 27377(1), which was normal in appearance. The 24 chromosomes, instead of showing the arrangement of twelve bivalents at metaphase I in pollen-mother-cells, were grouped into eight bivalents plus a circle of four and a chain of four chromosomes. It is with the chain of four chromosomes only that we are concerned in the present paper. There was only one unmodified 1 2 chromosome; the equivalent of the other 1·2 chromosome was found in a free fragment and a fragment which was translocated to another chromosome.

Presumably as a result of radiation treatment, the 1·2 chromosome was broken into two parts, the break occurring in the region where the spindle fiber is inserted.³ Crosses with appropriate testers have shown that it was the ·1 half which remained free as a rod-shaped chromosome. At

¹ Bergner, A. D., S. Satina and A. F. Blakeslee, 1933. Prime types in *Datura*. *Proc. Nat. Acad. Sci.* 19: 103-115.

² Bergner, A. D., S. Satina, A. G. Avery and A. F. Blakeslee. 1929. Translocated Sugarloaf, a pure-breeding chromosomal type in *Datura* induced by radium treatment. *Science* 70: 562.

³ Confusion has arisen because of the numerous senses in which the word "attachment" is used. Following Sharp and some of his associates, we are using the expression "insertion of the spindle fiber" instead of "attachment of the spindle fiber." This is equivalent to Belling's⁴ phrase "fusil attachment." The word attachment has also been used in regard to unions between non-homologous chromosomes (translocations) by such workers as Painter and Muller,⁵ Dobzhansky⁶ and Belling⁴; to unions

ovario omnino infero, vertice plano, stylo brevi obtuso, loculis 3; pyxidio cylindrico-trigono-conico (pedicello gracili 3 cm. longo), 8–9 cm. longo, pericarpio coriaceo subtenui (1–2 mm. crasso) extra rugoso longitudine 12–15-costato intus septorum 3 vestigiis pallidis signato, zona calycari 3–3.5 cm. diametro, vitta interzonali erecta 6–10 mm. longa, operculo subrugoso 3–3.5 cm. diametro plano leviter umbonato, columella crassa triquetra; seminibus circiter 12 a pyxidio compressis oblongis, 6.5–7 cm. longis, 1.5–1.8 cm. latis, scuto embryonifero centrali, ala membranacea cincto.

Maranhão: Maracassumé River region, Candido Mendes, *Froes 1763* (leaves and flowers); Mata da Cachoeira, *Froes 1901* (flowers and fruits). It is locally known as "Tauary branco," and the bark is said to furnish a fiber used for caulking boats.

SAPOTACEAE

Pouteria ovata A. C. Smith, sp. nov. Arbor, ramulis cinereis teretibus glabris rugosis; foliis glabris, petiolis gracilibus rugosis 12–20 mm. longis supra canaliculatis, laminis coriaceis nitidis ovato-oblongis, 9–14 cm. longis, 5–6.5 cm. latis, basi cuneatis, apice breviter acuminatis, margine integris anguste revolutis, costa utrinque prominente, nervis secundariis utroque 10–12 patulis prope margines adscendentibus utrinque elevatis, venulis reticulatis subtus prominulis; inflorescentiis axillaribus, pedunculo communi crasso 1–5 cm. longo densissime ferrugineo-sericeo, floribus tetrameris 6–10 in glomerulos aggregatis, pedicellis 2–5 mm. longis velut pedunculo pubescentibus; sepalis utrinque arctissime puberulis subaequalibus late ovatis, circiter 1.5 mm. longis et 2 mm. latis, exterioribus carnosius fuscis, interioribus pallidis; corolla membranacea extra parce puberula intus glabra 2–2.5 mm. longa, lobis tubum aequantibus orbicularibus circiter 1 mm. latis margine imbricatis; staminibus in medio corollae affixis, antheris subsessilibus ovoideis subacutis 0.7 mm. longis per rimas laterales dehiscentibus; staminodiis carnosius deltoideis acutis quam antheris paullo brevioribus; ovario depresso-globoso sub anthesi 1–1.5 mm. diametro pallide striguloso, loculis 2, ovulo in quoque loculo unico, stylo carnoso glabro 1 mm. longo; fructis cinereis glabris ellipsoideis, circiter 2 cm. longis et 1.5 cm. latis (pedicellis fructu ad 7 mm. longis), pericarpio 2 mm. crasso, semina unica.

Type, *Froes 1841*, collected Aug. 31, 1932, on high rocky land near sea coast, Ilha do Trauíra, Maracassumé River region, State of Maranhão. It is a species of the Section *Pseudocladia*, related to *P. lateriflora* (Benth.) Radlk. and *P. cladantha* Sandwith. From the former it differs in the shape of its leaves, which are decidedly broader in proportion and cuneate rather than acute at base, and in its shorter pedicels which give the inflorescence a more compact appearance. From *P. cladantha*, the new species differs by its long petioles and its more prominent secondary nerves, and by its glabrous differently shaped fruit.

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pedicellis velut pedunculis 10–20 mm. longis prope basin bracteolas lanceolatas 1.5 mm. longas mox deciduas gerentibus; sepalis 4 ovatis acutis, 7–8 mm. longis, 4–5 mm. latis, utrinque densissime arcte puberulis; toro 5 mm. diametro; staminibus numerosissimis quam calyce brevioribus, filamentis gracilibus 1 mm. longis dense setosis, antheris (appendiculis filiformibus ad 1.5 mm. longis inclusis) circiter 3.5 mm. longis parce puberulis; ovario ovoideo sub anthesi 3 mm. longo pilis ad 0.8 mm. longis densissime setoso, loculo unico, placentis 4 parietalibus ad margines interiores ovulos multos gerentibus, stylo gracili ovarium aequante glabro.

Type, *Froes 1918*, collected Sept. 16, 1932, at Mata da Cachoeira, Maracassumé River region, State of Maranhão. A local name is "Guaibiraba branca." It is related to *S. Garckeana* Schum., from which it is distinguished by the fine reticulate venation of the larger thinner leaves and by the short inflorescences. *S. Garckeana* has a much thinner flagellate anther tip and placentas completely fused to form a 4-celled ovary, while the placentas of the new species are quite separate at their inner edges.

LECYTHIDACEAE

Couratari coriacea Mart. Two collections of Froes are referred to this species, previously described from fruit only.¹ The pyxidium of Martius' original collection seems to agree perfectly with those of the present collection; it is exceptionally thin in texture, slender, and with an uncontracted mouth. The species is also noteworthy because of its obtuse leaves and the small number of stamens, which are arranged in a single row. The following description is based on the cited specimens:

Arbor ad 45 metralis, trunco ad 2 m. diametro; ramis ramulisque fuscis rugosis glabris lenticellatis; petiolis juventute supra puberulis et canaliculatis 10–15 mm. longis; laminis coriaceis siccitate fuscis oblongis, 6–9 cm. longis, 3.5–4.5 cm. latis, basi apiceque obtusis vel rotundatis, margine integris vel obsolete crenulatis, utrinque glabris, costa prominente, nervis secundariis utroque 10–13 patulis prope margines anastomosantibus supra planis subtus elevatis, venulis inconspicue reticulatis; inflorescentiis post defoliationem maturantibus racemosis ut videtur ramulos breves terminantibus, 3–9 cm. longis, rhachidibus arctissime puberulis, pedicellis gracilibus maturitate ad 12 mm. longis cum calycibus velut rhachidibus puberulis; calycis tubo obconico, sepalis 6 aequalibus carnosius ovatis, circiter 2 mm. longis et 3 mm. latis, basi connatis, apice rotundatis, margine fimbriatis; petalis aequalibus membranaceis juventute puberulis obovatis, circiter 2 cm. longis et 1 cm. latis; androphoro explanato 25 mm. longo, ligula 5–9 mm. lata, galea carnosa 8–10 mm. diametro appendiculis numerosissimis anantheris filiformibus imbricatis oblecta, staminibus circa annulum 10–14, antheris subsessilibus ovoideis 0.5 mm. longis;

¹ Berg; Mart. Fl. Bras. 14¹: 510, pl. 75, f. 2. 1858, Miers, Trans. Linn. Soc. 30: 283. 1875.

sis rugosis supra leviter canaliculatis 2–4 mm. longis, laminis oblongis vel obovato-oblongis, 8–13 cm. longis, 3.5–4.5 cm. latis basi acutis apice obtusis vel leviter emarginatis margine integris vel levissime crenulatis utrinque glabris, costa supra plana vel impressa subtus valde prominente, nervis secundariis utroque 7–10 patentibus supra leviter elevatis subtus prominentibus, venulis utrinque elevatis et conspicue reticulatis; paniculae in axillis superioribus solitariae 7–20 cm. longae ad anthesin tomentellae; flores 2–3 in glomerulos subsessiles conferti bracteis 2–3 chartaceis oblongis 3 mm. longis et 1 mm. latis extra dense pilosis intus glabris subtenti; pedicelli 1 mm. longi dense pilosi; sepala 5 subcarnosa oblonga 3.5 mm. longa et 2 mm. lata extra dense pilosa intus glabra; petala 5 praeter squamas glabra membranacea orbicularia, ungui gracili 1 mm. longo, lamina 2–2.5 mm. diametro rotundata basi 2-squamata, squamis adscendentibus subdeltoideis circa 1 mm. longis utrinque pilosis; discus carnosus annularis; stamina 8 intra discum, filamentis erectis carnosus circa 3 mm. longis infra medium pilis pallidis 0.8 mm. longis ornatis supra medium angustatis, antheris oblongis 1 mm. longis glabris; ovarium in floribus ♂ sterile subconicum 1–2 mm. longum dense strigulosum; sepala in fructu persistentia; capsula late pyriformis trialata purpureo-brunnea 15 mm. longa et 18 mm. diametro apice late truncata basi in stipitem brevem angustata, extra leviter pilosula praesertim ad basin, septis angustis glandulis minutissimis arcte notatis; semina atra ellipsoidea 7 mm. longa 4 mm. diametro.

Type, *Froes 1978*, collected Oct. 22, 1932, at Campo de Boa Esperança, Maracassumé River region, State of Maranhão. A local name is "Pão de Arapuce." It is most closely related to *C. racemosa* (Vell.) Radlk., in comparison with which it has subentire rather than serrate leaflets, larger flowers with a longer and paler pubescence, and petals which are more definitely orbicular, more slenderly clawed, and with rounded rather than subacute scales. Young ♀ flowers of the new species have not been observed.

ELAEocarpaceae

Sloanea reticulata A. C. Smith, sp. nov. Arbor ad 15 metralis, trunco 15 cm. diametro; ramis ramulisque teretibus cinereis glabris, juventute arcte puberulis et lenticellatis; foliis plerumque prope apices ramulorum congestis, petiolis gracilibus teretibus decidue tomentellis 1–3 cm. longis basi et apice incrassatis; laminis papyraceis glabris (juventute nervis parce tomentellis) ovatis, 10–18 cm. longis, 5–9 cm. latis, basi obtusis vel rotundatis saepe subcordatis, apice obtusis vel breviter acuminatis (apice ipso saepe emarginato vel calloso-mucronulato), margine undulatis, nervis secundariis utroque 6–9 patulis cum costa supra planis subtus prominentibus, venulis copiosissime reticulatis utrinque prominulis; inflorescentiis solitariis axillaribus quam foliis multo brevioribus (ad 6 cm. longis) cymulosis 3 (vel 2)-floris (floribus rare solitariis epedunculatis), pedunculis teretibus rectis 2–4 cm. longis tomentellis,

the same region is the same; its local name is "Camacary." Apparently only six petaliferous species of *Moquilea* have been described hitherto. Of these, five are sharply distinguished from ours by obvious characters. Specimens of the sixth, *M. elata* Pilger, have not been examined; according to the description it differs from ours in its leaves opaque on both sides and in its elliptic ciliate petals. It was collected on the upper Rio Acre, more than two thousand miles from Maranhão, and in all probability is not conspecific with *M. riparia*.

MELIACEAE

Trichilia Froesii A. C. Smith, sp. nov. Arbor ad 20 metralis, trunco prope basin 20 cm. diametro; ramis ramulisque cinereis striatis mox glabris; foliis 15–25 cm. longis, petiolis supra canaliculatis basi incrassatis 1.5–3 cm. longis, juventute arcte cinereo-puberulis mox glabris, rhachidibus velut petiolis vel subteretibus, foliolis plerumque 9 alternis, petiolulis incrassatis rugosis decidue puberulis 1–2 mm. longis (foliolis terminalibus ad 12 mm. longis), laminis subcoriaceis oblongis, 7–15 cm. longis, 2–5 cm. latis, basi cuneatis, apice obtuse acuminatis ad apiculum minutum callosum, margine integris vel subcrenulatis, utrinque fuscis praeter costam interdum puberulam glabris, supra nigropunctatis, costa supra leviter elevata subtus prominente, nervis secundariis utroque 12–14 rectis patulis prope margines adscendentibus supra planis subtus elevatis vel planis, venulis copiose reticulatis subplanis; paniculis multifloris axillaribus solitariis pedunculatis saepe folia subaequantibus, ramulis striatis cinereo-puberulis; floribus breviter pedicellatis, extra subtilissime cinereo-puberulis; calyce cupuliformi, dentibus 5 deltoideis obtusis; petalis 5 valvatis oblongis carnosissimis, circiter 3 mm. longis et 1.5 mm. latis, apice acutis incurvatis; filamentis connatis, tubo subcarnoso glabro 1.5 mm. longo, margine inter antheras dentes 0.5 mm. longos gerente; antheris 10 ovoideis 0.8 mm. longis; ovario sessili late conico, dense et arcte luteo-hirsuto, 3-loculari, loculis 2-ovulatis, ovulis collateralibus; stylo carnoso breviter cylindrico, stigmate truncato.

Type, *Froes 1917*, collected Sept. 16, 1932, at Mata da Cachoeira, Maracassumé River region, State of Maranhão. It is a species of the Section *Moschoxylum*, related to *T. japurensis* C. DC., from which it differs by having the leaflets black-punctate and with fewer secondary nerves, and the filament tube glabrous rather than puberulent within.

SAPINDACEAE

Cupania olivacea Gleason & Smith, sp. nov. Sect. *Trigonocarpus* Radlk. Frutex vel arbor parva; ramuli sulcati mox teretes, primum ferrugineo-tomentelli demum glabri; folia 15–20 cm. longa, petiolo subtereti 4–9 cm. longo basi incrassato primum tomentello mox glabro, rhachide petiolo simili, foliolis alternis 6–8 subcoriaceis supra sicco olivaceis subtus fuscis, petiolulis subcar-

arcuato-adscendentibus supra prominulis subtus elevatis, venulis reticulatis subtus prominulis; inflorescentiis solitariis axillaribus multifloris quam foliis brevioribus, ramulis angulatis densissime cinereo-tomentellis; floribus (in ramulis brevibus dense aggregatis) hermaphroditis extra arcte cinereo-tomentellis pedicellatis (pedicellis circiter 1.4 mm. longis) sub anthesi 2–2.5 mm. longis et 4 mm. diametro; perianthii tubo subnullo, lobis 6 subaequalibus oblongis intus glabris, 1.5–2 mm. longis, 1 mm. latis, apice obtusis; staminibus 9 fertilibus, antheris oblongis obtusis 0.4 mm. longis; serierum exteriorum filamentis glabris quam antheris paullo brevioribus; seriei tertiae filamentis prope basin glandulis binis sessilibus auctis; ovario glabro ellipsoideo sub anthesi 1 mm. longo, stylo carnoso ovarium fere aequante; bacca subglobosa ad 1 cm. diametro rugosa, cupula obconica carnosa margine integra.

Type, *Froes 1790*, collected July 16, 1932, on "terra firma" in the Maracassumé River region, State of Maranhão. A local name is "Louro do Igapó." It is a species of the Section *Mespilodaphne*, in which the angled branchlets, large leaves, close sericeous pubescence, and spreading oblong perianth segments distinguish it. It is allied to *O. Beyrichii* (Nees) Mez, which differs, in addition to the above characters, by having staminodes.

ROSACEAE

Moquilea riparia Gleason, sp. nov. Arbor 12 m. alta, ramis juvenilibus castaneis glabris lenticellis parvis notatis; petioli crassi rugosi minutissime puberuli 5–8 mm. longi; foliorum laminae subcoriaceae oblongae vel obovato-oblongae usque ad 11 cm. longae 6 cm. latae ad apicem apiculatam rotundatae ad basin rotundatae vel truncate vel paullulum cordulatae utrinque glabrae supra subnitentes subtus opacae, costa supra leviter elevata subtus prominente, venis secundariis in utroque latere 10–15 leviter arcuato-adscendentibus supra obscuris subtus paulum elevatis, venulis utrinque obscuris; stipulae jam delapsae; paniculae laterales vel subterminales 10 cm. longae, axibus cano-pubescentibus, ramis lateralibus 4–6, 2–6 cm. longis; flores numerosi in racemos spiciformes laterales et terminales dispositi, fasciculis plerumque 3-floris, bracteolis lanceolatis pubescentibus pedicellos 0.8–1 mm. longos vix aequantibus; hypanthium poculiforme vel late turbinatum 1.6 mm. altum 3 mm. in diametro extra breviter cano-pubescentis intus araneosum; sepala reflexa triangularia 1.1.2 mm. longa lataque cano-pubescentia; petala patentia suborbicularia 2 mm. longa fere glabra; stamina circ. 20 horizontaliter patentia, filamentis gracilibus glabris 2–3 mm. longis, antheris late ellipsoideis dorsifixis 0.5 mm. longis; ovarium centrale ovoideum 1 mm. longum dense araneosum, ovulis 2 a basi adscendentibus; stylus basalis gracilis 4 mm. longus fere usque ad apicem araneoso-villosus.

Type, *Froes 1961*, collected 20 Oct. 1932, in sandy soil in *terra firma*, Maracassumé River region, Maranhão. *Froes 1935*, from *varzea* land in

Plantae Krukovianae II

H. A. GLEASON AND A. C. SMITH

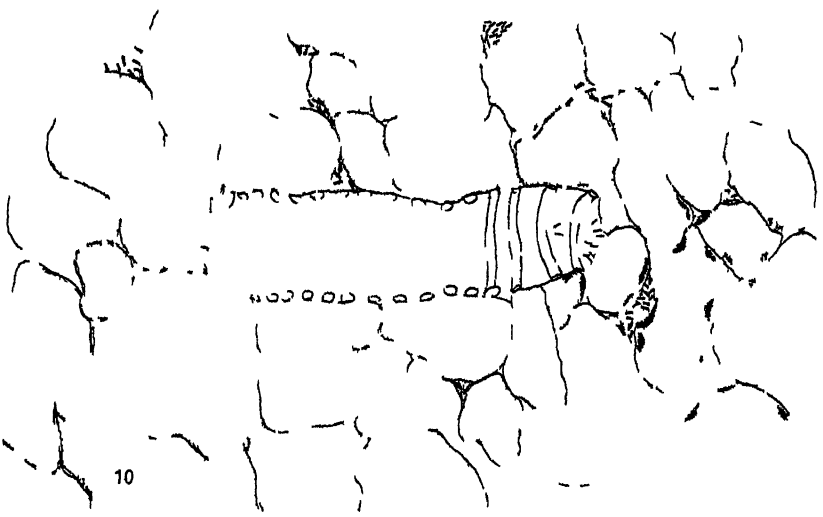
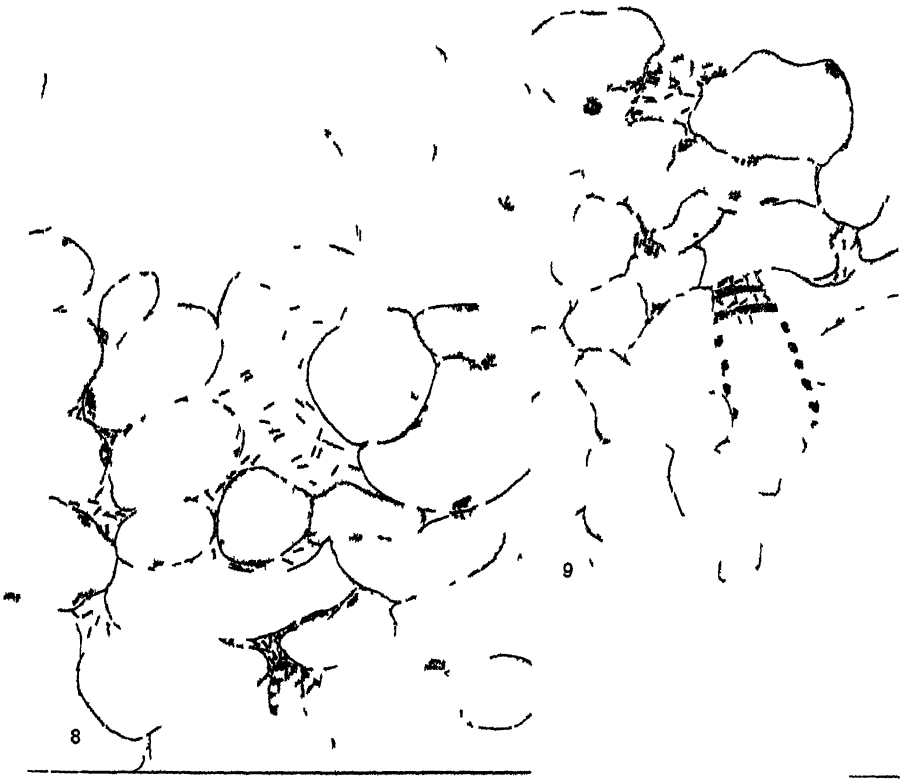
During 1932, several hundred flowering plants, mostly woody, were collected in the vicinity of the Maracassumé River, State of Maranhão, Brazil, by Mr. R. Froes, under the direction of Mr. B. A. Krukoff. The several new species disclosed by this collection, together with one previously incompletely known species, are described in the present paper. All types are deposited in the herbarium of the New York Botanical Garden.

LAURACEAE

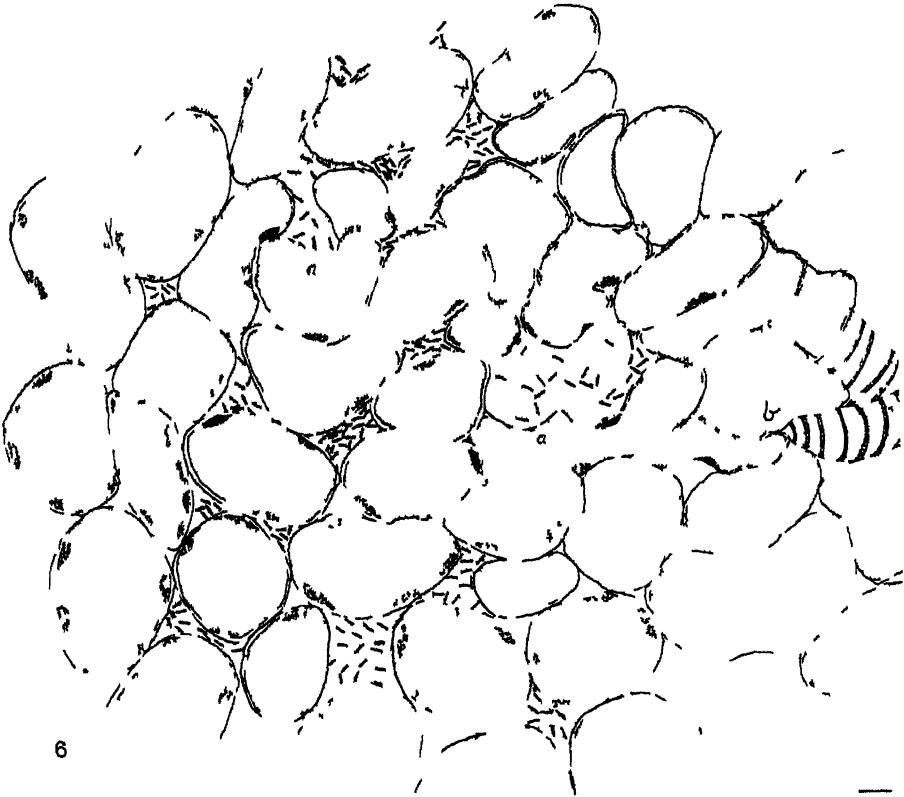
Aniba opaca A. C. Smith, sp. nov. Arbor ad 10 metralis; ramis ramulisque teretibus fuscis, juventute cinereo-puberulis mox glabris; petiolis crassis rugosis supra canaliculatis 6–11 mm. longis; laminis coriaceis glabris siccitate fuscis opacis oblongis, 12–18 cm. longis, 3–6 cm. latis, basi cuneatis, apice breviter acuminatis, margine integris et leviter recurvatis, costa utrinque prominente, nervis secundariis utroque 8–12 adscendentibus supra planis subtus prominulis, venulis reticulatis subplanis; inflorescentiis ad apices ramulorum congestis, ad 10 cm. longis et 30-floris, ramulis subteretibus et floribus cinereo-tomentellis; floribus pedicellatis (pedicellis 1–2 mm. longis) bracteolis parvis mox deciduis subtentis, maturitate circiter 3 mm. longis et diametro; perianthii tubo obconico lobos subaequante, lobis 6 aequalibus late ovatis obtusis pelucido-punctatis intus glabris, circiter 1.5 mm. longis et latis; staminibus 9 fertilibus, staminodiis nullis; serierum exteriorum filamentis glabris quam antheris paullo brevioribus, antheris late ovoideis obtusis, locellis minutis apertis; seriei tertiae filamentis basi glandulis binis sessilibus auctis, antheris subglobois; ovario glabro ellipsoideo sub anthesi 1 mm. longo, stylo rugoso ovarium aequante, stigmate subtruncato.

Type, *Froes 1730*, collected May 6, 1932, at Ubim, Maracassumé River region, State of Maranhão. A local name is "Louro Abacate" and the tree is used for lumber. It is related to *A. guyanensis* Aubl., than which it has shorter stouter petioles, fewer secondary veins, and larger flowers. It also bears a close superficial resemblance to *A. rosaeodora* var. *amazonica* Ducke, but has its leaves strictly glabrous rather than tomentellous beneath, and its ovary glabrous and comparatively long-styled.

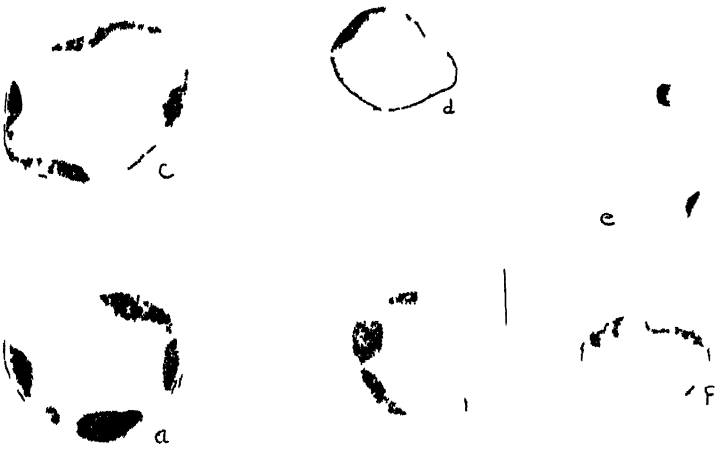
Ocotea Froesii A. C. Smith, sp. nov. Arbor ad 12 metralis, trunco ad 20 cm. diametro; ramis ramulisque crassis conspicue angulatis arcte flavo-cinereo-velutinis demum teretibus et glabris; petiolis crassis rugosis velutinis supra canaliculatis 8–17 mm. longis; laminis coriaceis siccitate fuscis ovato-oblongis, 15–30 cm. longis, 6–13 cm. latis, basi cuneatis, apice acuminatis, margine integris et leviter recurvatis, supra glabris subtus densissime et minutissime sericeo-tomentellis, costa subtus prominente, nervis secundariis utroque 5–7



MEIER BLACK ROT

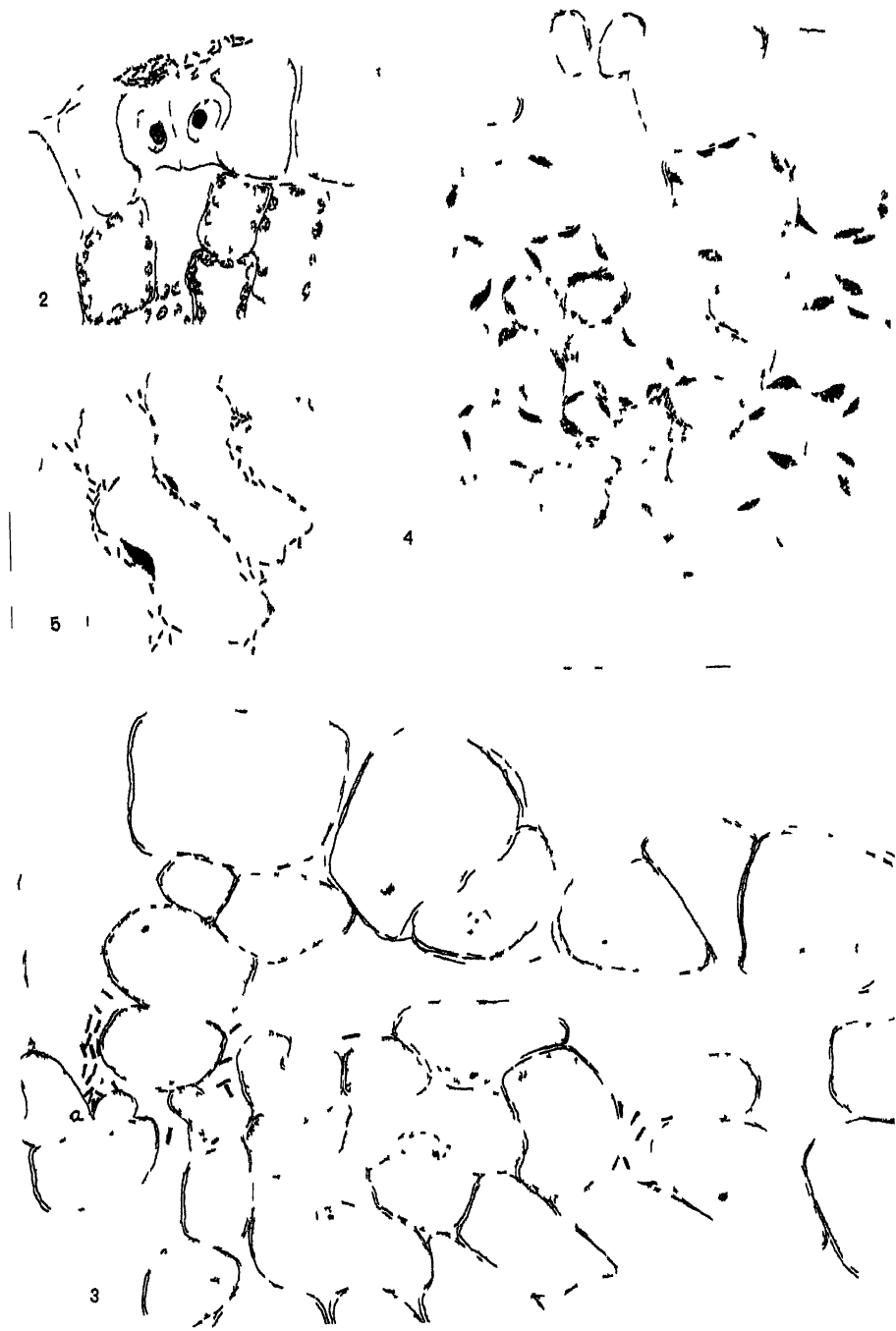


6



7

MEIER BLACK ROT



MEIER BLACK ROT



MEIER BLACK ROT

Explanation of plates 9-12

(All figures drawn with aid of camera lucida)

Plate 9

Fig. 1. ($\times 800$) Cross section of tooth of cabbage leaf showing

- a. water pore cavity
- b. specialized water pore or hydathode
- c. terminus of tracheid.

Plate 10

Fig. 2. ($\times 1200$) Bacteria lying in a film of blue-staining material above a stoma.

Fig. 3. ($\times 1600$) Cross section of hydathode region of cabbage leaf 3 hours after inoculation

- a. The bacteria are wedged between cells.

Fig. 4. ($\times 1200$) Bacteria among cells beneath pore cavity. $7\frac{1}{2}$ days after inoculation.

Fig. 5. ($\times 1200$) Cells of epithem region 6 days after inoculation. Bacteria have destroyed the middle lamellae and are present in the narrow passages between cells. The chloroplasts and nuclei have collapsed against the walls and in certain cells they have disappeared.

Plate 11

Fig. 6. ($\times 1200$) Bacteria are in the spaces between the cells

- a. wall of cell invaginated by bacteria
- b. terminus of tracheid.

Fig. 7. ($\times 1200$) a. Cell of uninfected plant from region bordering a pore

- b. Cell of uninfected plant from epithem region
- c. Cell from region bordering the pore of plant infected 6 days
- d, e. Cells from epithem region of plant infected 6 days
- f. Cell from region bordering pore of plant infected 3 hours.

Plate 12

Fig. 8. ($\times 1200$) Water pore region of tooth. Bacteria lie in the intercellular spaces of cells and are entering the tip of a tracheid. 6 days after inoculation.

Fig. 9. ($\times 1200$) Serial section to Fig. 8. Bacteria in the tracheid.

Fig. 10. ($\times 1200$) Serial section to fig. 9. Bacteria are in the tracheid.

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CONCLUSIONS

1. Infection of plants by *Bacterium campestre* takes place when a continuous pathway of liquid from the tips of the tracheids to the hydathodes is present.

2. Entrance of bacteria into the leaf is dependent on a combination of biological and mechanical factors. Motility of the organisms, diffusion, and convection currents may influence to a slight degree the entrance of the bacteria. Recession of drops of water contaminated with the bacteria from the hydathode when transpiration is resumed in the plant is doubtless an important factor.

3. In their passage from the hydathodes to the tracheid tips the bacteria travel only in the epithem region where a fluid pathway is present. They retain their motility and growth is in the direction of the food supply, which is the tracheid tips. Here also, however, passage of the organisms is facilitated by recession of the liquid material of the epithem region as water evaporates from the leaf. Their ability to secrete enzymes that dissolve the middle lamellae makes passage between cells possible.

4. The organism secretes products that give a blue color to the fluid of the intercellular spaces when stained with the triple stain. The fluid in which the bacteria lie is but slightly denser than the fluid of similar regions in uninfected plants.

5. The organism causes invagination of cell walls, disappearance of nucleoli, the collapse of nuclei and chloroplastids against cell walls, and a decrease in the amount of cytoplasm of the cells.

I am very grateful to Professor R. A. Harper, with whom this study was begun, for his stimulating interest and advice, to Professor J. S. Karling for his assistance and criticism, and to Professor S. F. Trelease for his helpful suggestions.

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this account it may be deduced that the fluid in which *B. campestre* lies is not exceedingly thick or jelly-like. It is none the less slightly denser than the liquid in the intercellular spaces, in the triple stain its limits are occasionally visible. Ordinarily, the liquid in which the bacteria lie completely fills the intercellular spaces. At least it does not precede the bacteria to any appreciable extent as is clearly evident where lamellae have been partially dissolved.

Smith reported that *B. campestre* forms a zoogloea in fluid exuded from water pores. I interpret this substance to be the same as that in which the bacteria lie within the cabbage plant. It is not dense enough to show convex tips and does not push its way between cells, as far as my observations go.

The bacteria have a very definite effect on host cells. In plants fixed three hours after spraying the protoplasts of cells with bacteria about them appear somewhat collapsed, and the outlines of the nuclei are difficult to follow. In cells 7 days after inoculation little cytoplasm can be seen. The nucleoli have disappeared. The nuclei have either collapsed against the walls or have disappeared. That liquids have been lost from the cells is evident from their lack of turgidity and invaginated walls. Plasmolysis is never complete, however, as the cytoplasm maintains its position around the cellulose walls. In certain cells a few bacteria are seen. I believe that they have been carried in by the knife when the sections were cut or that the walls have disintegrated after an infection of 6 days allowing them to enter. This effect of *B. campestre* on the host tissue is thus very similar to that reported for *B. amylovorus*, *B. tabacum* and *B. vignae* by the investigators noted above. *B. campestre* does not cause the abnormal formation of cells as described for *B. radicola* and *B. tumefaciens*.

Entrance to the tracheids appears to be at their spiral tips. Often the bacteria may be seen lying along the lateral wall, but they do not gain entrance to the vessel. A number of investigators have assumed that the bacteria dissolve their way through the tracheid walls by means of enzymes. Here, the bacteria without any great evidence of mass action appear to pass through the tips of the tracheids.

The action of *B. campestre* in the above study appears thus to be quite different from the infection of horse-radish by what is said to be a new variety of *Bacterium campestre* reported by McCulloch in 1929. The organism causes leaf spots of horse-radish, and less readily of cabbage and related plants. Infection is usually stomatal. The mode of infection and the action of the bacteria in the tissues as reported by McCulloch are very different from the action of the typical black rot organism in the cabbage plant.

One marked difference between my preparations and those of Smith is that I have never found the cavity beneath the pores filled with bacteria. They are always sparsely grouped with their long axes in various directions in spaces between cells just beneath the cavities and in the spaces further down toward the tracheids. The time required for the bacteria to pass from a pore to a tracheid is six or seven days, according to my observations.

The blue-staining material in which the bacteria lie, I believe to be the fluid of the intercellular spaces of the host plus enzymes and waste products perhaps that are given off by the bacteria. It is not characteristic of the bacteria in the cabbage tissue alone. It is evident in smears of organisms in cabbage broth, in distilled water, and on the surface of leaves which have been sprayed. It is therefore not entirely similar to the material that Bachman reports as being drawn from the cell sap in the pear tissue by *B. amylovorus*.

Doubtless enzymes and bacterial products tend to cause the liquid in which the bacteria lie to stain a blue color. The stain is denser about the organisms that are farthest advanced in the tooth, probably because these organisms are younger and more active than those nearer the water pore.

Nixon, Haber, Riker, Hill and others in studying the migration of *B. amylovorus* and *B. tumefaciens* state that the organisms move through the host tissues in the form of a zoogloea. That is, the bacteria secrete a jelly-like matrix about themselves, and the migrating mass of bacteria or the zoogloea pushes its way through the tissues with its blunt, rounded tip.

The question as to whether or not a zoogloea is present in *B. campestre* is relative, and depends to a certain extent, it seems to me, on one's definition of such a structure. It is impossible thus to draw a sharp line between those with and those without zoogloae. A zoogloea is generally defined as a gelatinous matrix secreted by the bacteria in which they lie. The consistency of the secreted substance is such as to make flagellar motion difficult or impossible. In the wide range of bacterial forms almost any degree of variation in the consistency of the gelatinous matrix may be found. The most extreme case is perhaps in the Myxobacteriaceae where the secretion is so copious that they are able to build definite symmetrical bodies. In forms such as *B. amylovorus* it is less abundant, and so on down the line to species like *B. campestre* where the secretion is comparatively slight. This latter organism does not lose its motility in the time it takes for the organism to reach the tracheids, as may be ascertained by examining hand sections of infected leaves. The bacteria move about in the spaces between cells so that their long axes point in various directions. They are not oriented in one direction as reported by Hill for *B. tumefaciens*. On

multiplication of the bacteria takes place in the direction of the tracheid tips. The organisms are seen densely aggregated in the epithem region where there is liquid between cells. They do not leave this region for the dryer, mesophytic part of the leaf.

In passing through the epithem region to the tracheid tips the bacteria appear to secrete enzymes which dissolve the middle lamellae, and they are thus able to pass between adjacent cells. As a rule, however, the cells in the epithem region are loosely placed in reference to each other. As a consequence the bacteria can doubtless travel for considerable distances without coming into direct contact with cells.

Riker and Ivanoff inoculated plants with India ink and dead bacteria and found that these substances appeared embedded in zoogloecae and moved at approximately the same rate and the same distance as the living crown gall organisms. In an attempt to determine whether or not liquids could enter the cabbage plants through the hydathodes and thus throw some light perhaps on the entrance of bacteria, guttating leaves of my material were soaked in amaranth dye. It was found that the dye entered only at the specialized pores at the teeth of the leaves from which a continuous pathway of liquid extended to the tracheid tips. The dye filled the entire tooth. It did not enter the stomata of the leaves where there was air between the cells. When leaves were soaked in India ink the cabbage juice precipitated the ink particles. There was no evidence that the ink particles together with the bacteria formed zoogloecae as described by Ivanoff and Riker in their work with the tomato. The cells in the epithem of the cabbage leaf are closer together with fewer intercellular spaces than in the tomato tissue studied, however.

Lack of moisture in the stomata, the substomatal cavities, and the intercellular spaces doubtless hinder and prevent the bacteria from entering the stomata on the leaf surface. Smith believed that infection could not take place through the stomata because of the waxy bloom on the leaves of cabbage plants. In various attempts that I have made to infect plants with the organism either by spraying with a suspension of the bacteria on washed leaves or by soaking leaves in a suspension of the organisms, no bacterial invasion was found to take place through the stomata. It seems probable to me that the tissue, although it offers many intercellular spaces, is generally too dry for optimum bacterial development.

This requirement of *B. campestris* for moist tissue is undoubtedly the reason for the scarcity of the disease in dry seasons as noted by earlier investigators. Riker, likewise, found that tumors of the tomato caused by *B. tumefaciens* develop only in the limits of tissue that has been water soaked by wounding or bruising.

disease develops in the region that is water-soaked. They conducted experiments with zoogloae in minute capillary tubes of various shapes to determine the effect of capillary attraction on the movement of this matrix and bacteria, and then attempted to apply the result in explanation of migration of the organisms in tomato stems. It is very difficult, however, to conceive from purely physical grounds how capillary attraction can operate when the intercellular spaces, as they report, are filled with liquid. Were they empty, or filled only with water vapor, the forces of adhesion and cohesion could readily operate and thus attract or draw fluids containing bacteria forward in the host tissues. At least capillary attraction does not seem to be of much significance in the entrance of the black rot organism in the water pores. As noted before, this occurs only when the intercellular spaces are completely filled and the excess water exudes in drops through the hydathodes.

Hill suggested that the fluid released in the tomato stem following the rupture of the cells would be reabsorbed into the surrounding tissue. Ivanoff and Riker claim that this would not be possible owing to the fact that the fluid contains the entire cell contents, not merely the cell sap. They suggest negative pressure as a cause of the rapid movement of the bacteria in the form of zoogloal strands in entering the tissue. Where the plants are infected by thrusting a needle through the stem on which the bacteria are placed entrance by this means is probable, due to the fact that the air in the stem is rarified. It could not, however, account for the method in which *B. campestre* enters the cabbage leaf through the hydathodes as there is present a continuous pathway of liquid from tracheid tips to pores before the leaves are sprayed with the bacteria, and also because the progress of the bacteria is slower than if negative pressure were operative. Three hours after inoculation the bacteria have been observed among the cells directly beneath the cavity of the hydathodes. They are not in a dense zoogloal form as reported for *B. amylovorus* and *B. tumefaciens*, but are embedded in a material that stains light blue and which takes the shape of the intercellular spaces.

From the study of freehand sections of the living leaf it is evident that the bacteria retain their motility as they pass from the hydathodes to the tracheid tips. This motility appears to be a more or less darting movement in various directions. As near as can be determined from living, unstained sections, the movement is not definitely oriented in the direction of the tracheids. I have never observed flagella on bacteria in stained slides of infected cabbage leaves.

Multiplication of the bacterial mass or colony appears to be in the direction of the food supply. As the food is exhausted near the hydathode,

diseased areas. Smith stated that the bacteria grow in the fluid extruded from the water pores during periods of high humidity and move in through the latter in the direction of the food supply.

The experiments which I have carried on confirm the view that a moist atmosphere is necessary if the bacteria are to enter the leaf and bring about infection. Infection took place only when plants had been placed under bell jars and drops of guttation water had collected at the teeth of the leaves before the plants were sprayed. The air in the roof greenhouse where the plants were grown is rather dry and for this reason natural infection of plants was difficult. Cauliflower plants, badly infected in the seedling stages, recovered and produced flowers without any indications of black rot.

The entrance of bacteria into the leaf is dependent upon a continuous pathway of liquid from the tips of the tracheids to the water pores when the plants are very moist and when transpiration is low. It is highly probable under such conditions that the bacteria which may be present at the teeth of the leaf may get into the water pores by their own motility.

If the drop of water at the pore remains for a long time diffusion may possibly aid to some extent in the entrance of bacteria and inert material, but since diffusion rates are generally so slow it is doubtless of little significance alone. Convection currents due to a difference between the temperature within the leaf and the outside air may also be a factor; but in view of the fact that the bacteria have been found considerably advanced in the tissues of the leaf in three hours, it is doubtful that diffusion and convection currents play a prominent rôle in the entrance of the organism.

Entrance of bacteria into the leaf is dependent on a combination of mechanical and biological factors. When evaporation through the stomata is checked due to excessive moisture of the air, surplus water that would ordinarily pass through the stomata in vapor form fills the spaces between cells of the epithem region and appears as droplets at the teeth of the leaves. Bacteria come into contact with this fluid. As the moisture content of the air gradually decreases, transpiration through the stomata is more actively resumed. The drop of water begins to evaporate, its borders begin to recede somewhat in the same fashion as a lake of water drying up, and as the recession continues some of the bacteria may be carried with it towards the pore and finally inside, while others are left stranded behind.

Riker and Ivanoff in experiments involving the entrance of crown gall bacteria into tissues from a wounded surface state that it is possible that capillarity, convection currents, and negative pressure are important factors. They state that due to wounding of the cells in the stem the inter-cellular spaces are filled with liquid material from the injured cells and the

deeper lying regions of the tooth than immediately beneath the pore cavity.

The organisms maintain their motility in the host tissue during the early infection stages. This was ascertained by examination of sections of the living leaf cut parallel to the surface. The bacteria moved in various directions with quick, darting motion made possible by the one-polar flagellum. Although motion was not in any one definite direction it was less hampered and more free at the periphery of the group.

Bacteria have not so far been observed within host cells in these early infection stages, except in a few cases where the leaves have been infected for some time, as is shown in figure 8 where the leaf was inoculated six days previously. In this case there are a few bacteria in a cell beneath the pore cavity. The wall of the cell is hard to define and it is probable that the bacteria gained access to the cell through the ruptured wall or were carried into it by the knife when the sections were cut. Walls of the cells may be invaginated and the nuclei have for the most part become so flattened against them that they are difficult to distinguish from the chloroplasts in fixed and stained preparations (fig. 7e).

Figures 8, 9, and 10 show serial sections of the same leaf in which the bacteria extend from the intercellular spaces beneath the pore cavity into a tracheid. The bacteria are not crowded in the intercellular spaces, and their long axes are pointing in various directions. In the region immediately above the tracheid the bacteria are definitely oriented so that their long axes are directed down the tip of the tracheid.

The next serial section of this same leaf shows a few bacteria in the broader section of the tracheid (fig. 9). In the third section (fig. 10), the bacteria are in the region near the apex of the tracheid. The organisms are so distributed that their individual forms are readily apparent. Nearest the apex they are oriented with their broad surface parallel to the sides of the tracheid, but farther down they are less definitely oriented and their long axes face in various directions.

DISCUSSION

It was early affirmed by Harding and Smith that infection of cabbages by the black rot organism takes place quite naturally by the bacteria entering the specialized water pores at the teeth of the leaves during the damp summer weather. The early investigators all emphasized the importance of moisture in infection and spread of this disease. Garman, who first observed infected plants, noted that periods of high temperature and excessive moisture are favorable for the organism. Pammell also found that plants infected with the organism in rainy weather recovered in dry weather, principally by means of the formation of a corky layer around the

to push their way between host cells with blunt advancing tips. In places the bacteria appear wedged between cells so that adjacent cells are forced apart (fig. 3a). The blue-stained material is not distinguishable in advance of the bacteria in these regions as in the case of forms with zoogloaeae.

At this time it is apparent that cells with masses of bacteria about them are slightly affected. The cytoplasm appears slightly plasmolyzed, the nucleoli have disappeared from the nuclei, and nuclei and chloroplastids have collapsed in the cytoplasm against the walls. The cell contents stain less densely than formerly. With a view of bringing out more specifically the host and parasite relationship at this stage I have drawn in figure 7 cells from the epithem regions of infected and uninfected plants.

The rate of the bacteria in entering and passing through the tissue varies. In many preparations of leaves sprayed 6 days before fixing bacteria have been found widespread throughout the entire tooth and can be traced from the water pores to their entrance into the tracheids. In other preparations, on the other hand, bacteria are present in great numbers between cells but have made less progress. Figure 4 shows bacteria among cells of a leaf that was sprayed $7\frac{1}{2}$ days before fixing. The organisms have not reached the tracheids, but are present in great abundance among the cells just beneath the pore cavity, filling the intercellular spaces completely. The homogeneous blue-staining material in which the bacteria lie is hard to distinguish here because of their great number. At least it is not visible in advance of the bacteria as described by Nixon, Haber, and Nabelek for *B. tumefaciens*, and Zaumeyer for *B. flaccumfaciens*, *B. medicaginis*, and *B. phaseoli*. At this time the bacteria appear definitely oriented with their long axes parallel and their ends directed toward the intercellular spaces which lead to the interior of the epithem region. The organisms are densely aggregated about the cells where the lamellae are still intact. It is difficult to distinguish between nuclei and chloroplastids in the cells that have bacteria about them. The nuclei, with no visible nucleoli, and the chloroplastids, appear collapsed in the scanty layer of lightly staining cytoplasm that surrounds these cells.

In preparations with bacteria in the intercellular spaces throughout the entire epithem region, the middle lamellae have been destroyed, and where the intercellular spaces are narrow the bacteria lie with their long axes parallel to the cell walls (fig. 5). In certain regions cells surrounded by bacteria appear to have lost their turgor and the walls have collapsed (fig. 6a). In these particular regions and also in the large intercellular spaces the bacteria are not crowded together and definitely oriented. The blue-staining homogeneous background in which they lie is much denser in the

rows of chlorenchyma cells. They are loosely packed together with large intercellular spaces below the upper, but appear to be closer together above the lower epidermis of the leaf. The large water pores of the epidermis of the tooth, or hydathodes, lead into cavities which are similar in structure but larger than those beneath the stomata (fig. 1a). In fixed material the water pores are always open. The width of the pore varies from 1.5μ , as is shown in figures 3 and 4, to 6μ as in figure 1b.

The tracheids do not follow a straight path but curve in and out among the chlorenchyma and epithem cells and end with one or two tips directed toward the hydathodes (fig. 1c, 6b, and 8). The spiral thickenings of lignin are connected with thin, anastomosing strands, and the cellulose portions of their walls appear thinner than the wall of the epithem cells which surround them.

Under normal conditions the water which is not used in the metabolism of the plant evaporates from the stomata. When the plant is kept very moist, however, and evaporation is lessened by reason of the high relative humidity of the air, drops of water appear at the teeth of the leaves. During such periods of high relative humidity, when there is a continuous pathway of liquid from tracheid tips through the water pores, infection with the black rot organism normally takes place.

Description of leaves sprayed with the bacteria. Fixed and stained preparations of inoculated cabbage leaves show that the bacteria enter only at the hydathodes. Bacteria were observed variously oriented in a film of blue-stained material on the epidermis and over stomata on leaves sprayed 7 days previously, but they did not gain entrance into the leaves in these regions. The film in which they lie appears sufficiently thick and consistent to support the weight of the bacteria, and extends about 6μ above the surface of the leaf, as is shown in figure 2. This same blue-staining material is also present in stained smear preparations on slides. The substance that stains blue appears to be secreted by the bacteria as soon as they are placed in sterile distilled water.

The first visible evidence of the bacteria in the pore cavities has been found 3 hours after spraying, as is shown in figure 3. In some preparations bacteria lie in the spaces between cells that border the cavity. They are stained red and lie in a thin film of blue material. In certain regions they are scattered loosely about in the intercellular spaces without any particular orientation. Between other cells they are packed more closely together, so that it is difficult to distinguish individual organisms. The blue material in which they lie fills the intercellular spaces completely. It none the less appears less dense than the zoogloae of *B. amylovorus* and *B. tumefaciens* figured by Nixon, Haber, Hill, Riker, and Ivanoff, and reported

areas below the nodes with bacteria and pricking deeply with a needle. Controls were stabbed with a sterile needle.

In order to observe the progress of the bacteria in the tissues permanent mounts were made. Portions of the margins of the leaves approximately .5 by .5 cm. were removed and fixed immediately after inoculation and at intervals of one to 24 hours and every succeeding day for 10 days. Flemming's weak, strong, and medium, Merkel's, and Allen and Wilson's modification of Bouin's killing solutions were used for fixation. The latter solution proved to be the most satisfactory. The material was embedded in paraffin and sections were cut 7-10 μ thick. The stains used were iron-alum haematoxylin and Flemming's triple. Both stains were satisfactory but the triple stain was of particular value owing to the differentiation between the host and parasite.

An effort was made to make flagella stains of the bacteria in the tissues at different time intervals, but so far this has not succeeded. Sections of the teeth of the leaves were cut parallel to the surface and these sections were mounted in water for study of the motility of the organisms. The progress of the organisms in the stem and the effect brought about on the host cells as a result of stab inoculations was watched by means of hand sections as well as by permanent mounts.

OBSERVATIONS

The progress and invasion of bacteria in most hosts are dependent to a certain degree on the compactness and continuity of the tissues through which they travel, particularly when moving in large zoogloal strands as in the case of *Bacillus amylovorus*. It is therefore essential at the outset to give a brief histological description of the structure of a normal uninfected cabbage leaf with special reference to the intercellular spaces, sub-epidermal chambers, cell contacts, and continuity with the vascular system.

The surface of the epidermal cells of the leaf is covered with a thick coat of cutin which prevents water from collecting in large drops on the leaf. In longitudinal and stained sections of the cabbage leaf, the tooth or terminus of tracheids appears thicker than the adjoining regions, owing to an abundance of small epithem cells which surround the apices of the tracheids as is shown in figure 1. The epithem cells are loosely arranged in relation to each other, with many intervening intercellular spaces.

They are frequently completely free of chloroplastids or at most contain only one or two. The cytoplasm, with nucleus embedded in it, appears as a thin layer just inside the cell wall.

Immediately beneath the upper and lower epidermis are three or four

by which the bacteria and inert substances move through the intercellular spaces is not definitely understood, but they suggest that capillarity and negative pressure are important factors.

The contention that certain bacteria exist in both filterable and non-filterable states has recently been advanced by Kendall, Sherman, and Safford (1931). Kendall maintains that *B. typhosus*, *B. coli*, and *Streptococci* when grown on suitable protein medium were rendered filterable, but by suitable procedure could be regained again in non-filterable form. Sherman and Safford report the presence of filterable forms of bacteria in various sources including milk and hay. They believe that these "primitive" types are probably present in greater numbers than the familiar forms of bacteria.

The formation of zoogloecae by three organisms parasitic on the bean, *B. flaccumfaciens*, *B. medicaginis*, *B. phaseoli*, was reported by Zaumeyer (1932). The organisms were embedded in a slimy matrix with high absorptive power which enabled them to rupture cell walls and cause the disintegration of cells.

MATERIALS AND METHODS

The plants used in this study were chiefly the Danish Ball Head and Flat Dutch varieties of cabbage and Best of All cauliflower, which were grown in the greenhouse at Columbia University. At the time of inoculation they varied in size from seedlings with two or three leaves to mature plants about eight months old.

The organisms used for the inoculations were obtained from the U. S. Department of Agriculture. The pathogenicity of the bacteria and the susceptibility of the plants to the disease were previously tested by stab inoculations into healthy plants and subsequent isolation of the black rot bacilli from the blackened veins which developed. The organisms recovered from these plants were then grown for two or three days on potato agar before inoculations were made.

As reported by early investigators infection in the field takes place through the teeth of the leaves when drops of guttation water are present. Accordingly an attempt was made in this study to simulate these infection conditions in the greenhouse as nearly as possible. Plants were placed under bell jars until drops of water appeared at the teeth of the leaves and then sprayed with a heavy suspension of bacteria in distilled water. Five to ten minutes were occupied in spraying the plants in this manner. They were then covered again with the bell jars for several hours and finally exposed to the air of the greenhouse.

Inoculations were also made directly into the stems by smearing small

teria, found that a chemotactic response to expressed tomato sap makes it possible for *B. tumefaciens* to enter surface wounds. He found that the galls which develop as a result of bacterial invasion coincide in outline with the water-soaked region formed by wounding the tissue. The bacteria are distributed in the liquid of the intercellular spaces.

In recent years the majority of investigators have emphasized the gelatinous matrix or zoogloea as the means of movement of bacteria through the plant tissues. The ability of *B. amylovorus* to form this zoogloea in fluid cultures was first noted by Arthur in 1886, and Smith in 1896 showed that *B. campestre* will also form a zoogloea in fluid cultures. In 1904 Peirce stated that the root nodule bacteria enter cells by softening or dissolving the cell walls. They move or grow through the opening, multiply rapidly, and form a thread-like zoogloea which grows chemotropically through the cells toward the nuclei.

Bacterium tumefaciens is figured by Robinson and Walkden (1923) as showing zoogloelial strands that intrude along the intercellular spaces and proto-xylem strands of infected plants. This work was confirmed by Hill in 1928 and studied further by Hill, Brittingham, Gibbons and Watts in 1930. Nabelek (1930) found these organisms traveling through the tracheae and between cambium and conducting cells of the geranium. He reported that the zoogloea dissolve the middle lamellae and that the organisms cause hypertrophy of cells and the metaplasie of cells into secondary tracheal elements at the wound surface.

Nixon (1927) found that *B. amylovorus* migrates through the tissues of water sprouts of the apple in the form of a zoogloea. Migration is intercellular in early stages. The matrix of the zoogloea is jelly-like with finely granular or homogeneous structure. The bacteria cause plasmolysis of the protoplast which leads to its collapse. Haber, in her study of *B. amylovorus* in leaf tissues of the apple in 1928, and Wahl in his work with the organism in the quince confirm the work of Nixon.

Pseudomonas pisi forms an "abundant slime" having "high absorptive powers" in the tissues of the pea, according to Skoric (1927).

Although Hill (1928) observed that motion of *B. tabacum* through the intercellular spaces of the tobacco is due to a zoogloea he stated that the zoogloea appeared to do little or no harm to the host cells.

B. vignae, according to Beach (1928) migrates through the intercellular spaces of the lima bean in zoogloelial form, and brings about disintegration of cell contents.

Ivanoff and Riker (1930) contend that purely mechanical means are influential in the migration of bacteria through the host tissues. By their studies of *B. tumefaciens* in the tomato they conclude that the mechanism

the progress of the bacteria towards the vessels is slow,—probably due to insufficient water supply or inability of the bacteria to dissolve the cell walls immediately and thus pass from the water pores to the veins. He stated that the juices of the parenchyma are not so well adapted to the needs of the organism as the fluid inside the vessels. Lack of sufficient aeration was the cause, in his opinion, of the slow sidewise movement of the organism in the stem, which thus prevents destruction of the plant.

Smith believed that the bacteria probably enter the vessels by pits or thin places in the wall.

While Smith was carrying on his experiments with the black rot organism Russell was likewise making a study of the disease, and in his paper published in 1897 stated that the bacteria develop in drops at the teeth of the leaves and by their motility work their way in through the water pores.

Hecke (1902) is in agreement with Smith when he states that the movement of *B. campestris* in the tissues is due to growth, not self motility.

Jones (1909) claims that “aside from the moisture content, susceptibility to infection is largely, if not wholly, dependent on the nature of the middle lamella.” Forty-five strains of bacteria causing black rot in species of *Brassica* were found to secrete an enzyme that dissolves the middle lamella. Complete solution of the cellulose and diastatic action were found to be lacking.

The importance of moisture mentioned by previous writers was also stressed in a short account of the life history of the black rot organism by Coulter, Barnes, and Cowles, 1910, who attribute the entrance of the bacteria into the tissues to mechanical means. Bacteria that are lying on the surface of leaves come in contact with the fluid of guttation given off at the hydathodes when evaporation is checked. This fluid is resorbed when transpiration begins again in the leaf, and the bacteria by contamination of the hanging drop pass into the chamber beneath the pore. Here they develop and “kill the adjacent cells, whence they enter the xylem and work backward, killing and rotting the bundles.”

Differing to some extent with the previous work of Burrill and Arthur on *B. amylovorus*, Bachman (1913) stated that the bacteria in apple tissue are surrounded by sap from the cells. This film of liquid does not precede the bacteria to any extent. She claimed that the host cells die because of loss of water, and that cell walls were ruptured by physical means or by osmotic pressure.

For a number of years investigators had attempted to stain plant tissues infected with *Bacterium tumefaciens*, the causal organism of crown gall in tomato and other plants. In 1923 Riker, working with these bac-

of the disease when they turned brown, he stated that the organisms might be able to pass into cells in an ultra-microscopical condition, or perhaps in the so-called germ condition they could, amoeba-like, creep through the spaces between the molecules. He found that the bacteria caused the disappearance of starch in the cells, although the remainder of the protoplasm was apparently not affected by them.

The work of Burrill on *Bacillus amylovorus* was followed by that of Arthur (1886). He stated that bacteria first attack the starch of the cells, then the cellulose walls, until finally "the whole tissue becomes a liquid mass." He found that in the living plant the organism produces "a mucilage or gum, which is soluble in water,—with the disengagement of carbon dioxide."

In 1891 Garman first noticed a disease of cabbages where the infected plants showed black veins and yellow leaves. By observations throughout the entire season he came to the important conclusion that the organisms are able to "invade and break down the tissues of the plant" only during periods of high temperature and excessive moisture.

The isolation of the bacterium causing the black rot of cabbage was actually accomplished by Pammell (1893–1895), who, as noted before, isolated a motile, yellow organism from the turnip and called it *Bacterium campestre*. He confirmed the work of Garman when he found that the characteristic yellowing of the leaf parenchyma and the blackening of the veins is checked by the dry weather of September, and made the interesting discovery that plants infected with the organism recovered by the formation of a corky layer about the diseased portions.

With the establishment of the fact that bacteria are the causes of plant diseases and are of considerable economic importance to plant growers, more detailed studies were begun with an attempt to find how the bacteria gain entrance to the tissues, and how they travel through the different parts of the plant. It was first maintained that progress of the parasite through the host tissue occurs by multiplication of the organisms in the direction of the food supply and by the motility of individual organisms.

Erwin F. Smith made detailed studies of *B. campestre* during the years 1897–1911. He found that turnips, cabbages, and many members of the genus *Brassica* were infected by the organism through the water pores at the teeth of the leaves. He stated that the bacteria grow in the fluid extruded from the water pores and move in through these pores away from the light toward the direction of the food supply. Entrance through stomata was impossible, in his opinion, because of the waxy bloom on the surface of the leaf which prevents the surface from becoming moist. He found that

it; and by mechanical means through which the bacteria are passively carried through the tissue.

As to the relation between parasite and host tissue it has been shown by various investigations that the action of different types of bacteria is not always similar. Certain organisms are normally found within cells and others are intercellular parasites. For example, *Bacillus amylovorus* that causes fire blight of pear is reported by Nixon to pass between the cortical cells of the twig. *Bacillus radicola* passes through the interior of the cells of the roots of clover and brings about hyperplasia of cells. *Bacterium tumefaciens* is an organism that is reported to be intercellular but it also brings about abnormal production of cells. *Bacterium campestre* is an intercellular parasite until it gains entrance to the tracheids. From then on the life of the organism is spent within the water vessels, and the death of the tissue is due to thrombosis, or the shutting off of water and mineral supply from the uninfected vessels below.

Various theories have been advanced as to the manner in which organisms enter cells, whether they dissolve openings in the cellulose walls; pass through pits or thin places in the walls, as suggested by Smith; whether in a minute form they are able to squeeze through spaces between molecules, as advanced by Burrill; or whether there is a filterable stage in the life history of bacteria during which they diffuse through the cell walls, as maintained by Kendall, Sherman, and Safford.

The present investigation was undertaken primarily to trace the progress of *B. campestre* from its first appearance in the pores at the teeth of the leaf to its entrance into the water vessels to determine how the bacteria enter the plant, their manner of progress through the host tissue, the effect of the bacteria on the host tissue, and to compare its method of migration with that of other bacteria causing plant diseases.

REVIEW OF LITERATURE

The literature dealing with bacterial diseases of plants in general is an extensive one and has been amply reviewed by E. F. Smith and other members of the U. S. Department of Agriculture and by other investigators of this country and Europe. I shall consider only such papers as relate to the problems of infection, progress of the bacteria in the host tissue, method of migration, and host and parasite relationship.

It is agreed by most investigators that bacteria have a destructive influence on the contents of the cells that they surround. This was proven first by Burrill (1881), one of the pioneer plant pathologists, who studied the relations of *Bacillus amylovorus* to the pear. He believed that the bacteria could enter cells, but since walls appeared unaltered until the late stages

A cytological study of the early infection stages of the black rot of cabbage

DOROTHY MEIER
(WITH PLATES 9-12)

INTRODUCTION

The fact that bacteria are the cause of certain plant diseases has been known since the latter part of the nineteenth century. The first extensive pathological work with the cabbage dates back to 1893 when Pammell upon examining turnips, *Brassica campestris*, with yellow, withered leaves and blackened veins, suspected the presence of bacteria and succeeded in isolating motile, yellow organisms from the plants. He termed this organism *Bacterium campestris*.

The organism was found to be causing great financial losses among cabbage and turnip growers in the middle west soon after Pammell's discovery, and the interest of phytopathologists of the state and national departments of agriculture was aroused. Detailed studies of the characteristics of the organism, the mode of infection, and its course through the host tissue were made as early as 1896 by Smith and Harding.

The size of the bacilli vary when isolated from different parts of the plant. When first introduced into the plant from agar cultures it varies in size from $5-7\mu$ to $4-5\mu$. When crowded in the vessels of the host it often loses its motility and becomes smaller and resembles a micrococcus.

It has been found that the organism enters the plant by means of the specialized water pores at the teeth of the leaves or by means of insect bites. The affected plants show blackened veins and ultimately dry, yellow leaves, and the path of the parasite can be traced by the blackened vascular strands in the leaves, petioles, stems, and roots. The disease is essentially a dry rot, but in the field it is often followed by invasion of secondary organisms which cause a wet rot with strong odors of decay.

The more recent investigations of bacterial diseases of plants have been primarily concerned with the problems of the manner and mechanism by which the organisms travel in the host tissue, their course in the plant, host and parasite relationship, and the manner in which they gain entrance to the cells. In general four different hypotheses have been offered as to how the organisms advance in the host tissues: growth by reproduction of the bacteria in the direction of the food supply; motility of the individual organisms; a gelatinous secretion or a zoogloea which pushes its way through the intercellular spaces of the host and carries the bacteria with

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Spirodela oligorrhiza collected in Missouri

ALBERT SAEGER¹

(WITH PLATE 13)

A species of Lemnaceae, *Spirodela oligorrhiza* (Kurz) Hegelm., not previously reported from North America, was collected by the writer in one of the slow-flowing streams in a wooded section of Swope Park, Kansas City, Missouri (U. S. A.), on Nov. 2, 1930. A portion of the stream which had been dammed by a fallen log was covered with thousands of specimens. *S. polyrrhiza* (L.) Schleid. was also present in small numbers. The two species could be distinguished easily by differences in size, shape, venation, and number of roots per plant. The identification was made from the description in Hegelmaier's monograph (1868, revised 1895). No other species of the genus other than the common *S. polyrrhiza* has to the writer's knowledge been described from North America. Palisot (1816) mentions *S. thermalis* from Virginia, but Schleiden (Hegelmaier 1868, p. 156) found it to be *S. polyrrhiza*. Bravo (1930) reports only *S. polyrrhiza* from Mexico. Hegelmaier (1878) states that only *S. polyrrhiza* is indigenous in Brazil. Thompson describes a second species of the genus for South America, *S. punctata* (Meyer) Thompson, based upon specimens in the United States National Herbarium which were collected in 1839 at Orange Harbor, Tierra del Fuego. His description and the accompanying figure do not correspond with the plants collected in Missouri. Recently Koch (1932) described a new species of *Spirodela* from Montevideo, Uruguay, *S. intermedia* W. Koch, which differs from both *S. polyrrhiza* and *S. oligorrhiza*, especially in the position of the roots (up to 18 in number) with respect to the basal bract.

Some of the specimens collected were pressed for the herbarium, and others were maintained in an aquarium for further study. Since the identification of certain Lemnaceae in the vegetative state is very difficult and uncertain on account of the extreme variation resulting from differences in environment, it has become my practice to cultivate newly collected specimens of doubtful identity in a synthetic nutrient solution (Saeger, 1925, 1933). In this way the development of vegetative characters in the different species is conditioned by an environment initially the same for all species under examination. Cultures of eleven species have been maintained for comparative study: two of *Spirodela*, six of *Lemna*, and three of

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Wolffia. In this group *S. oligorrhiza* may be identified readily, even in the vegetative state.

Hegelmaier reported *S. oligorrhiza* from Australia, India, Java, and Japan. Recently Lancaster (1930) reported it from New Zealand. It is not likely that this species has been overlooked in the past by botanists in this country. It may have been introduced recently. It was again collected by Dr. F. H. Woods in September, 1932, in southwest Missouri, from a pond containing goldfish. Inquiry revealed that the plants in this pond had been obtained from a supply house in Ohio, but no information could be obtained about the original source of the plants. No doubt *S. oligorrhiza* has become established in other localities and will be found by other collectors. It is one of five species I used in a study of the relation of manganese to nutrition. A photograph of this species may be found in this publication (Saeger, 1933, plate 9). This species has produced flowers on several occasions while growing in pure culture.² Since flowering of the Lemnaceae is of special interest (Saeger, 1929; Hicks, 1932), the conditions which induced this flowering are being studied.

A brief description of the species is given, so that others who collect similar specimens may readily identify it. Hegelmaier's excellent monograph (1868, rev. 1895) should, however, be consulted for additional information.

Spirodela oligorrhiza (Kurz) Hegelm.

Plants oblong-obovate, strongly asymmetric, base bluntly cuneate, apex obtusely pointed, united into colonies of from 2 to 10. Plants 4 mm. long \times 2.6 mm. wide or smaller, their size varying with growth conditions. A nodal embryonic point occurs about one third the distance from base to apex, from which arise 2 to 6 adventitious roots, 5 to 6 veins, and 2 vegetative points. Buds develop from these vegetative points to form the daughter plants. A basal stalk connects the daughter plant with its parent. In very young plants a basal bract occurs, divided into a dorsal and a ventral portion, the latter extending over the node, and later being punctured by all the outgrowing roots. This structure becomes almost obliterated when the plants have reached maturity. (It is not found in any species of *Lemna*, but a similar bract occurs in *S. polyrrhiza*.) Roots 3 cm. long or shorter, with sharp-pointed root cap. A row of papules prominent on upper surface along midvein. A sac-like spathe encloses two stamens and one pistil. Stamens about 1 mm. long, the one nearest the base ripening a day before the second one. Anthers 2, transversely dehiscent. Pollengrains globose or nearly so, spinulose, about 0.018 mm. in diameter. Pistil about half the length of the stamens, both curving upward

² Methods of obtaining Lemnaceae in pure culture have been described by Saeger (1930), Clark (1931), and Hopkins (1931).

from the edge of the leaf. Pistil flask-shaped, with funnel-shaped stigma. Seeds not observed. Upper epidermis with numerous stomata, lower epidermis and adjacent cells usually pigmented. Rhaphides and druses present in many cells throughout the plant.

No specialized resting or "winter" plants were observed sinking to the bottom of the substrate (as in *S. polyrrhiza*), but the plants became very small under poor growth conditions. This species is distinguished readily from *S. polyrrhiza* by its smaller size, fewer roots and veins, and more oblong leaf; and from *Lemna minor* (which it resembles in size) by the presence of more than one root per plant and by its more asymmetric shape. In pure culture, the flowers appeared after the exhaustion of certain nutrients in the water, but no fruit was developed. The vegetative growth of plants that are in bloom is retarded, and such plants are smaller (2.6 mm. \times 1.4 mm.) than those actively growing.

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Explanation of plate 13

Figs. 1-15. *Spirodela oligorrhiza*.

Figs. 1 & 2. Dorsal view of colonies showing venation, roots, and method of budding.

Fig. 3. Ventral view of plant in flower. *a* spathe. *b* basal stalk. *c* node. *d* daughter plant. *e* flap of bud pocket. *f* midvein. *g* root (severed). *h* bud pocket, flap of bud pocket removed.

Figs. 4 & 5. Young plant about 1 mm. long, dorsal view, showing part of basal bract. *a* point of attachment to parent plant. *d* dorsal portion of bract.

Figs. 6 & 7. Young plant about 1 mm. long, ventral view, showing part of basal bract, young roots, and buds. *a* point of attachment. *b* & *c* young buds developing into daughter plants. *e* young roots just before piercing the bract. *f* ventral portion of bract.

Fig. 8. Distal portion of root. *a* root cap.

Figs. 9, 11, 13 & 14. Floral parts emerging from edge of plant.

Fig. 10. Old, partially dried stamen.

Fig. 11. Young pistil appearing before stamens.

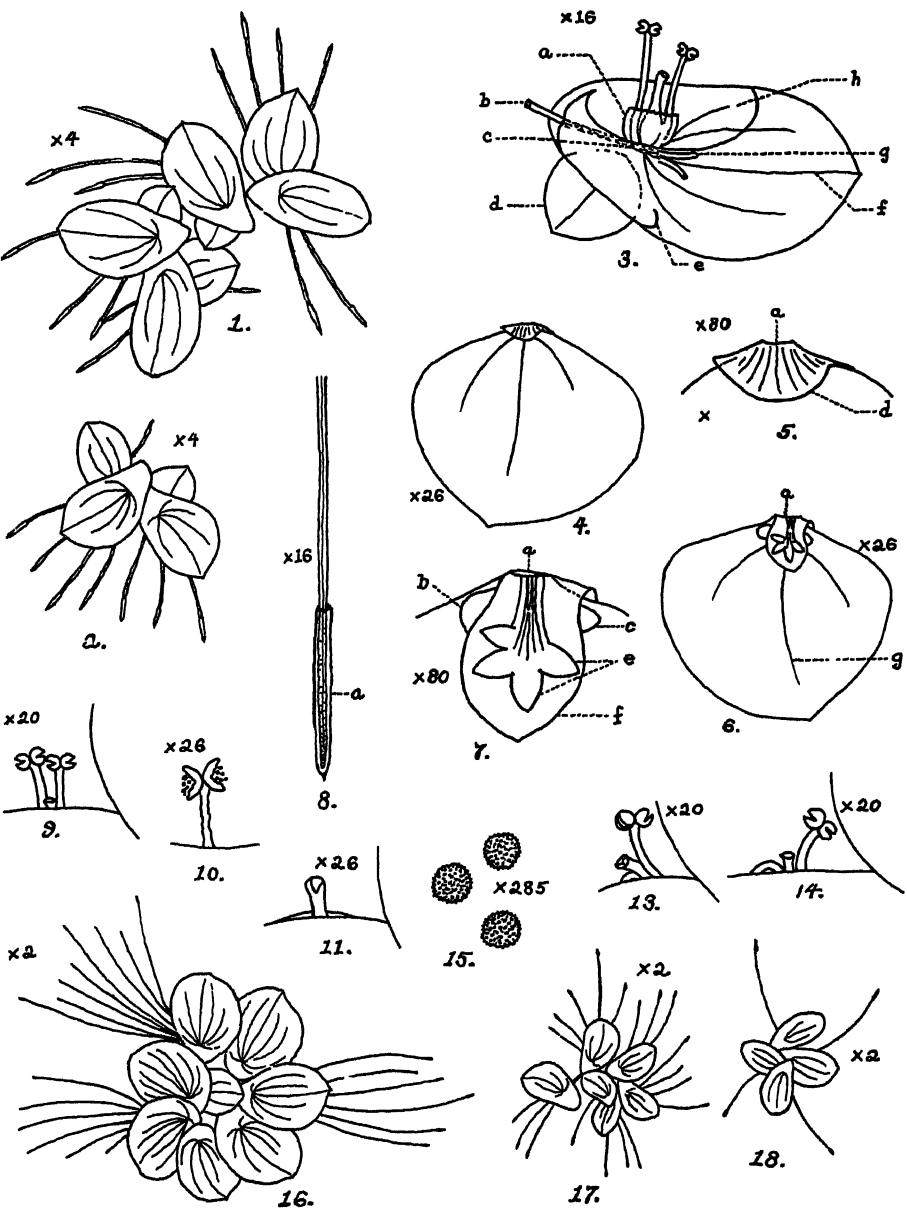
Fig. 15. Pollengrains.

Figs. 16-18. Comparison of three species of Lemnaceae.

Fig. 16. Colony of *Spirodela polyrrhiza*, roots shown on three of the plants.

Fig. 17. Colony of *S. oligorrhiza*.

Fig. 18. Colony of *Lemna minor*.



SAEGER SPIRODELA OLIGORRHIZA

Pleistocene plants from Cuba

EDWARD W. BERRY

(WITH PLATE 14)

Included in the paleobotanical material from Cuba gotten together by the late Arthur Hollick and turned over to me by the New York Botanical Garden are two lots from the Pleistocene which are of especial interest.

The first of these was collected by Barnum Brown in 1911 and came from the celebrated hot spring of Chapapote at Baños de Ciego Montero in Santa Clara province and found in association with a Pleistocene mammal fauna.¹ The plants from this locality were subsequently identified by Percy Wilson of the N. Y. Botanical Garden and the woods by Prof. Saml. J. Record of the Yale School of Forestry. They comprise the following:

Pinus caribaea Morelet (3 cones)

Juniperus sp. (wood)

Mimusops emarginata (L.) Britton (fruit)

Conocarpus sp. (wood)

The second lot came from an asphalt pit 100 meters west of the Hamel well about 3 km. east of Sabanilla de las Palmas in Matanzas province and was collected early in 1933 by Doctors Dickerson, Bermudez and Richards of the Atlantic Refining Co. It comprises a number of pieces of asphalt-impregnated wood the identification of which has not been attempted, a considerable number of various sized fruits of *Spondias lutea* L. and a single fruit of *Chrysobalanus icaco* L.

Such of the foregoing as require comment will be taken up in the accepted systematic order.

PINUS CARIBAEA Morelet (figs. 14, 15)

There are three cones in the collection—a fairly complete unopened specimen, an opened specimen, and a third badly macerated specimen. The first two are figured. These were determined by Percy Wilson, and there can be no doubt regarding the identification. The species is supposed to occur near the coast of the United States from South Carolina to Louisiana, in the highlands of Central America and in the Bahamas, Cuba and the Isle of Pines, a geographical distribution, which if correct, is indicative of considerable antiquity for the species.

There is some confusion in the precise limits of the recent forms which have been referred to this species but this has no bearing on the present occurrence.

¹ Brown, Barnum. 1913. Some Cuban Fossils. Amer. Mus. Jour. 13: No. 5: 221-228.

The *Juniperus* wood from Chapapote spring presumably represents *Juniperus barbadensis* L., the common Bahaman and West Indian red cedar.

CHRYSOBALANUS ICACO Linné (fig. 11)

A single specimen of a drupe of this species is contained in the collection from the asphalt deposit. The modern species, which is widely distributed from southern Florida through the Antilles to northern South America, is essentially a coastal species. Leaves referred to the genus *Chrysobalanus* have been described at a number of geological horizons in various regions, but the only one of these that is absolutely beyond question is *Chrysobalanus eocenica* Berry² from the lower Eocene of southeastern North America, which is represented by both leaves and characteristic fruits.

SPONDIAS LUTEA Linné (figs. 1-10)

The most common fossils in the asphalt deposits are the fruits of this species. They are of all sizes and conditions of preservation due to the peculiar configuration of the stone of the drupe. This is irregularly ligneous, that is, its configuration is one of irregular conical elevations separated by rounded excavations. The stone is covered in life with a fibrosuberous filling, which may shrink on drying and fissure in a wholly irregular way, but which in the majority of cases shrinks transversely much more than longitudinally and splits into regular segments that give it the appearance of a tardily dehiscent capsular fruit. This condition is shown in figure 2 of a fossil fruit and in figure 8 of a recent fruit. With more or less maceration the outer coat becomes more or less eroded and this condition is shown in figure 1 of a fossil fruit and figure 7 of a recent fruit. Sections of fossil fruits showing two seed cavities are shown in figures 4-6 and similar sections of recent fruits are shown in figures 9 and 10. These sections also show more or less clearly the line of contact between the stone and the overlay. I cannot detect any differences in form or structure between the fossil and the recent fruits of *Spondias lutea* except that some of the fossils are more prolate, as in figure 4, than any recent fruits I have seen in a rather large series from different places.

The only other living species of *Spondias* in the Antillean region (*Spondias purpurea* L.) has fruits which average not more than two-thirds the diameter of those of *Spondias lutea* L. They are also much more prolate and almost twice as long as they are wide. The stone is less excavated. The flesh appears thinner and less suberous and dries to a wrinkled condition

² Berry, E. W. 1930. U. S. Geol. Survey Prof. Paper 156: 73, *tf.* 13, *pl.* 12, *figs.* 2-4.

with no trace of the capsular-like shrinkage cracks which develop in *Spondias lutea*. It is true that I have seen only three specimens of the fruits of *Spondias purpurea*, and it may be that in a larger series all of the differential features enumerated above might not hold, but in any event the fossil fruits are very obviously different from those of this species.

The fruits of *Spondias lutea* are the most common objects in the beach drift throughout the Antilles and on both coasts of tropical America and the species also occurs in inland situations as well as in coastal situations. From the structure and occurrences as described above it follows that these fruits are very buoyant as Sloane pointed out in the late 17th century. It has not been found in the European drift although Guppy determined experimentally that it would float for at least 7 months. Viability is lost, however, after 2 or 3 months. Guppy seems to imply that the genus reached equatorial America from tropical West Africa but this does not seem to me to be the case.

The genus contains 6 or more existing tropical species all but one of which, *Spondias mangifera* Willdenow of the Indomalayan region, are represented in America and at least three of these are confined to America. Fossil species are rare. A leaf referred to this genus has been described from the Pliocene of eastern Brazil³ and related fossil fruits have been described from the Old World Eocene⁴ and Miocene⁵ under the generic name *Spondiaecarpum*.

The occurrence of the fruits in the Pleistocene of Cuba sheds no certain light on whether the situation was coastal or inland, but the degree of erosion of some of the specimens and their association with *Chrysobalanus* rather favors a coastal interpretation.

MIMUSOPS EMARGINATA (Linné) Britton (fig. 13)

This species is represented by a transverse section of one end of the fruit with the features shown in figure 13. This came from the Chapapote spring, and I do not know how it happened to be cut or by whom, but it was presumably complete when collected. It shows several developed and traces of abortive seed cavities as well as the histology of the pulp. Undoubtedly the microscopic features could be made out if the fossil were sufficiently old or unique to repay the effort.

The recent form is a rather small tree found in dry situations and essentially a strand type. It ranges from southern Florida and the Bahamas

³ Hollick and Berry. 1924. Johns Hopkins Univ. Studies in Geology, 5: 74, pl. 7, fig. 9.

⁴ Langeron, M. 1899. Soc. hist. nat. d'Autun, 12: 453, pl. 3, figs. 2, 4.

⁵ Menzel, P. 1913. Jahrb. k. preuss. geol. Landes. 34: 6, pl. 1, figs. 8-18.

to Cuba and will doubtless be discovered eventually on other Antillean islands, since if the fossil is correctly determined it is a species of considerable antiquity. A rather similar species, *Mimusops sieberifolia* Berry,⁶ occurs in southeastern North America as early as the lower Eocene.

SUMMARY

All of the described species, representing the families Pinaceae, Rosaceae, Anacardiaceae and Sapotaceae are still existing and common elements in the same region in which they occur fossil, so that they afford no evidence of changes in specific features or changes in geographic distribution which might indicate great antiquity. On the other hand the physical changes which took place in the Antillean region since late Tertiary times can hardly be regarded as sufficient to have brought about great changes in the flora; and these plants, like those I described some years ago from Trinidad,⁷ could very well have existed in early Pleistocene times as well as at any subsequent time.

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⁶ Berry, E. W. 1916. U. S. Geol. Survey Prof. Paper 91: 339, *pl. 99, figs. 2; pl. 100, fig. 3*.

⁷ Berry, E. W. 1925. U. S. Natl. Mus. Proc. 66: Art. 21.

Explanation of plate 14

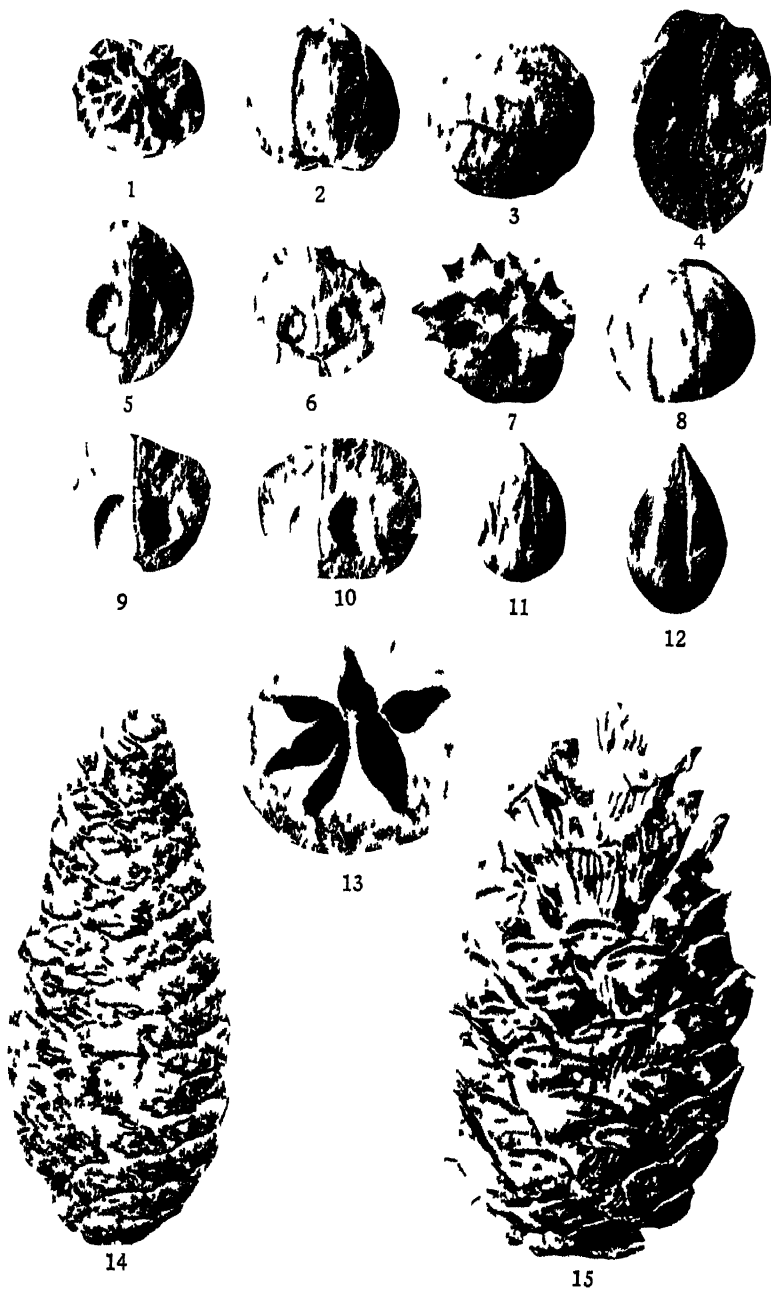
Figs. 1-10. *Spondias lutea* Linné. 1-6. Pleistocene. 7-10. Recent.

Figs. 11, 12. *Chrysobalanus icaco* Linné. 11. Pleistocene. 12. Recent.

Fig. 13. *Mimusops emarginata* (Linné) Britton. Transverse section of fruit.

Figs. 14, 15. *Pinus caribaea* Morelet.

All natural size.



The so-called "chemical stimulation" of *Aspergillus niger* by iron, zinc, and other heavy metal poisons

ROBERT A. STEINBERG

The rapid accumulation of papers dealing with the nutrition and stimulation¹ of *Aspergillus niger* had led to a condition, prior to the introduction of the method of nutrient purification, that made it almost impossible to decide upon the authenticity of much of the data and its interpretation. Since an increased interest is again being shown in this field and in similar studies with green plants, a few observations on recent developments might be of aid in helping to clarify the situation. A brief discussion of what is meant by stimulation with respect to chemical action is also included since so much confusion exists regarding its meaning that it has even been recommended by Kostytschew (1931. 2: 327) that the term be no longer employed.

Limitations of space forbid discussion of the many papers in this field excepting to note that the majority of the earlier investigators adopted the view of Pfeffer that the effect of heavy metal salts on the fungus is due to partial poisoning or "chemical stimulation." In 1919, however, Steinberg succeeded, by means of a nutrient purification method which he developed, in obtaining such large increases in growth of *A. niger* with zinc and iron that the theory of "chemical stimulation" with respect to their action was no longer tenable. The data also, for the first time, gave really convincing evidence of the indispensable need of a fungus for zinc and for iron. Inhibition of spore formation could not, moreover, be attributed solely to zinc since it was shown that the increased acidity of the nutrient solution brought about by the fungus also participated. Also both growth and the effectiveness of zinc in aiding growth varied with the acidity. Tests with other "stimulants" gave negative results. The author, therefore, considered he had proved both iron and zinc indispensable for the fungus; and assumed the growth increases reported for other so-called "chemical stimulants" as also due to causes other than "chemical stimulation."

Beginning with 1927, Steinberg's data and technique, which had so far received but little attention, obtained ample confirmation through the work of Bortels, Roberg and Metz, whose data will be referred to subsequently. These investigators also came to the conclusion that iron and zinc are indispensable for the growth of *A. niger*; and that copper also seems necessary.

¹ "Stimulate" and its derivatives, when employed in this paper with reference to increase in vital activity by poisons, will be so indicated by quotation marks.

While "chemical stimulation" or "stimulation" has reference in this field specifically to increases in vital activity of an organism in the presence of sub-lethal doses of a poison, the same expressions are also used with other meanings. To stimulate means ordinarily, "To arouse to or to increase action in, by applying some form of stimulus"; without implication of inhibition or reference to nutrition. Many have used the term as synonymous with "increase."

The meaning assigned to these terms and their derivatives by Pfeffer (1893, p. 75) is as follows:—"Reizung liegt vor, wenn der von aussen oder von innen ausgehende Anstoss die Veranlassung gibt zu *irgendwelches* Aktionen, welche mit den im Organismus disponibeln oder erreichbaren Mitteln ausgeführt oder betrieben werden. Durch die Reizung können ebensowohl Tätigkeiten neuerweckt oder auch die schon vorhandenen Aktionen in neue Bahnen gelenkt werden." Stimulation, according to this concept, may be due to either internal or external stimuli and may manifest itself as either excitation or inhibition. The stimulant furnishing the stimulus does not supply any material or energy (i.e. act as a nutrient) in bringing about the response; although Pfeffer (1895) states elsewhere that nutrients may also furnish stimuli, as may a variation in concentration.² Stimulation may consist in arousing an activity anew or the original activity may be modified. The responses are usually but not necessarily such as are normally associated with the plant. While the stimulant furnishes no energy in bringing about stimulation, it must, evidently, require the expenditure of some energy, even if entirely disproportionate in magnitude to the response, to bring about the internal change or stimulus in the organism from which the response develops. A relation exists between degree of stimulus and magnitude of response.

"Chemical stimulation" refers specifically to those phenomena (Pfeffer 1895) in which, "... andere Thätigkeiten des Stoff- und Kraftwechsels werden offenbar durch kleinen Mengen . . . verschiedener, besonders auch giftiger . . . Körper *beschleunigt*. Theilweise dürfte sich um physiologische Gegenreactionen handeln . . . bei schädlichen und anderen Eingriffen hervorrufen können"; catalysis, he states, might also play a part. Since Pfeffer (1897) also includes iron, it is evident that the "stimulant" may be either essential or non-essential. "Chemical stimulation," then, is any excitation in vital activity of an organism caused by toxicity. The increases are of presumably abnormal nature since due to abnormal condi-

² Increased growth through increased concentration, though ample material for the nutrition of the organism is already present, should be considered as a response to stimulation.

tions. The term does not include inhibition nor nutritive effects. The expression, toxicity, is generally used with reference to stimulative effects of inhibition.

The phenomena referred to usually as ion toxicity are seldom spoken of as "chemical stimulation" nor the compounds employed as "stimulants" or poisons. Nevertheless studies in this field do not have to do only with metabolism (unessential materials are also effective) but deal more especially with external chemical stimuli affecting the permeability and other regulatory mechanisms of the protoplast. Since the stimulus may be either excitation or inhibition; and since the stimulative effects of all substances and of concentration are included, "chemical stimulation," and chemical toxicity may be considered as limited phases of ion toxicity studies. It should be remembered however that in "chemical stimulation" it is assumed that the increased vital activity is caused by toxicity and is abnormal, whereas in ion toxicity it is assumed that increased vital activity is caused by elimination of toxicity and is normal.

The organism has been pictured as a system in unstable equilibrium, which, though it be in balance with its physical and chemical environment, is subject to internal stimuli, continually appearing in their normal sequence even in a constant environment, and to external stimuli; an extraordinarily complicated system of regulatory stimuli and metabolic responses. It might therefore prove advisable, in order to avoid confusion, to encourage the use of the terms, excitation and inhibition, with reference to stimulative or non-metabolic causes (Reize) only; just as the terms anabolism and catabolism, are used with reference to nutrition or metabolism (Stoff- und Kraftwechsel). It must be emphasized also that use of the term, stimulation, definitely implies a non-nutritive regulatory impulse that may result in normal as well as abnormal responses; whereas "chemical stimulation" refers to abnormal excitation only.

In the case of *A. niger* and zinc, which have served as the classic materials for the study of "chemical stimulation" for 63 years, the following reasons have been advanced for concluding that the increased growth is abnormal or "stimulative," instead of normal or nutritive. The fungus seems to make good growth and form spores in the absence of zinc. Many poisonous substances, cobalt, nickel, cadmium, iron, etc., have been reported to bring about similar growth increases. The growth increases found are relatively small—10 to 300 per cent. Spore formation is unfavorably affected. Lastly the cultures form "heavy, opaque and wrinkled" felts in the presence of zinc, whereas when zinc is omitted the felts are "translucent, slimy and smooth."

It is a mistake, to being with, to assume that "stimulants" *must* cause

inhibition of sporulation when they serve to aid growth. There are no specific data showing this to be a fact. Furthermore, there is much evidence showing that even with zinc there need be no inhibition of sporulation. Steinberg (1919) obtained results indicating that high concentration of magnesium sulphate (or traces of iron and copper which it might contain) and increased concentrations of iron phosphate counteract the harmful action of both zinc and high acidity upon sporulation. Also that ammonium hydroxide acts similarly, presumably by preventing an increase in acidity of the nutrient solution generally occurring in the presence of zinc. Results leading to the same conclusions have been reported by Bortels (1927), Roberg (1928, 1931) and Metz (1930). Roberg has done much to clear up this phase of zinc "stimulation" by recognition of the elimination by iron of the harmful effect of both zinc and acidity upon sporulation.

The fact that only a moderate increase in growth took place when poisonous substances were added to the nutrient solution has also been accepted as proof that toxicity and not nutrition is the underlying cause of these phenomena. Data already in the literature will demonstrate however that this could only be true because of incomplete removal of heavy metals from the nutrient solution in the researches upon which this statement is based. Steinberg (1919, Exp't 36) found that in a complete nutrient solution containing zinc, the addition of iron may increase the growth of *A. niger* 4370 per cent. Similarly zinc when added to a complete nutrient solution containing iron may increase the growth of *A. niger* 230,900 per cent. Increases in dry-weight of these magnitudes can hardly be termed "stimulation."

Furthermore the objection to the nutritive interpretation, that good growth (about 300 mg. dry-weight) occurs in the absence of "stimulants" from the nutrient solution, can no longer be retained in the case of iron and zinc. The growth on an "iron-free" purified solution amounts, according to Steinberg (1919, 1920), to but 36.5–67.5 mg. dry-weight, that in a "zinc-free" purified solution to 0.5–12.5 mg. dry-weight. These figures refer to actual growth after deducting 3.5 mg. dry-weight of the inoculum. Comparing these figures with a maximum possible yield of about 1200 mg., it must be admitted that growth is almost entirely suppressed when iron or zinc is omitted.

Mention may be made at this point of the fact that in the presence of zinc the felts of *A. niger* become "opaque, heavy and wrinkled." These morphological changes have also been cited by many as evidence of abnormality and therefore "stimulation." It would seem sufficient to point out however that if zinc is essential for its growth, the felts of *A. niger* should become heavier when zinc is added, and probably more opaque

because of better nutrition. As the result of experience with this fungus, the inclination would be to associate the formation of "translucent and slimy" hyphae with lack of growth due to poor nutrition and to injury by chemicals. Similar hyphae, for example, are always found at the bottom of the culture flasks where oxygen is lacking. Often these conditions are associated with inability to form a felt upon the surface of the nutrient solution. The wrinkling of the felts is also probably due to improved nutrition.

Due to the many contradictory results obtained through the use of standard methods, only data obtained with a purified nutrient solution will be considered here in relation to the increase in growth or "stimulation" reported for many poisonous substances. In so doing one minimizes at least any likelihood of errors due to contamination of the solution, although of course increases due to other causes may still, if not carefully examined, be mistakenly held due to toxicity. Zinc is absent from the purified solution, moreover, to a degree never before attained. Nevertheless, under these conditions, which may result in an increased growth with zinc of about 230,000 per cent, not one of a total of eleven poisonous substances recently tested have been found capable of causing "stimulation." But one of the above substances, copper, has been found to increase growth appreciably and then only if zinc is also added. Copper, since it cannot be replaced by other elements and because it seems to be necessary for formation of black pigment in *A. niger* spores, is assumed to be essential. Poisons do not, it is evident, increase growth in the complete or almost complete absence of zinc nor, excepting copper, in the presence of an optimum concentration of zinc.

No increase in growth could be obtained by Steinberg (1919, 1920), using a purified nutrient solution, with sodium silicate, and salts of cobalt or uranium. Bortels (1927) could not, moreover, obtain "stimulation" of growth when using the method of nutrient solution purification with sodium silicate, arsenic, or salts of cobalt, manganese, aluminum and mercury either in the presence or absence of zinc nor Roberg (1928) with boron, iodine, arsenic or salts of uranium, manganese, and cadmium. Of the 14 poisonous substances listed as "stimulants" by Steinberg (1919), on the basis of data in the literature, it is to be noted that the most consistent and recent data indicate three (iron, zinc, copper) are essential, and seven (silicon, cobalt, uranium, manganese, cadmium, aluminum, mercury) are incapable of "stimulating" growth. Four (beryllium, nickel, lithium, fluorine) still remain to be tested by the method of nutrient purification.⁸ Specificity such as exhibited by iron, zinc and copper is char-

⁸ Since this paper was written the author has obtained experimental evidence demonstrating

acteristic of the biologically essential elements and seems to be dependent upon the limited range in properties of the elements that may successfully be used in the complicated system of reactions comprising the protoplasm.

In "chemical stimulation" it is assumed that with a progressive increase in the supply of a "stimulant," for example iron, growth is increased through nutrition until the optimum is reached. Above this point, however, is supposed to be another region of increasing growth due, not to nutrition, but to toxicity. Only above this second maximum does growth always show a decrease with further increase in iron. Growth in the nutritive range is considered normal, in the "stimulative" range morphologically abnormal. The ability of a "stimulant" to cause increased, if abnormal, growth in an optimum solution is definitely implied.

These growth increases have also been associated with *changes in balance of functions* whereby the direct inhibitory effect of toxicity on one function of the organism (sporulation) results in a secondary excitation of another (growth). Toxicity according to this view results in inhibition of reproduction, the materials conserved furnishing additional nutriment for an increase in mass. Excitation therefore need not enter at all into the increase in mass. This theory affords no explanation, however, as to the reason for non-utilization of much of the available nutriment in the so-called "normal" or "un-stimulated" culture, and its effective utilization in the "stimulated" culture.

Taking into consideration further the large number of possible "stimulants" and their wide differences in chemical constitution it does not seem plausible to postulate the existence of any growth mechanism sufficiently complex to be capable of bringing about the identical, or closely identical, responses known as "chemical stimulation" through individual reactions. The same growth mechanism must function in its orderly and normal processes with or without "stimulation." The probability is also that the presence of these heterogeneous materials within the cell could result in similar action only through destruction of normal cell constituents and their oxidation through normal respiratory processes. Since the increase in respiration by poisons has been reported to effect an increase in the economic coefficient with *A. niger*, thus conserving materials for growth in weight, a general destruction of cell materials need not, however, be considered. The data, moreover, discussed by Palladin (1923), indicating that for increased respiration through action of poisons, the cell must remain intact and that the enzymes concerned do not increase in effective-

the necessity of manganese, as well as of iron, zinc, and copper, for the nutrition of this fungus. None, however, of the other chemical elements just enumerated (including beryllium, nickel, lithium and fluorine) gave an increase in growth.

ness, would also tend to indicate that these phenomena are colloidal and take place at the boundary of the cell as in ion toxicity effects on permeability. Destruction, for example, of only a minute layer of the plasma membrane by "stimulants" so as to increase permeability to oxygen or carbon dioxide, or both, might lead to slight increases in growth through a more thorough decomposition of respiratory materials and conservation of a larger balance of plastic materials for growth in mass. The belief in similar increases in mass with toxicity is in the case of *A. niger* based on what now appears to be erroneous evidence. Increased acid production in the "stimulated" culture is also contrary to this hypothesis. There is no need, therefore, to assume the growth mechanism of the cell provides for stimulatory, catalytic, and other effects ("Gegenreactionen") matching in number the possibly innumerable and diversely constituted "chemical stimulants"; nor that conservation of material for growth in mass occurs.

A direct and positive test for "chemical stimulation" of growth is possible with an optimum nutrient solution for growth, since an increase in growth through any addition cannot under these conditions be ascribed to nutrition (essential substances or partial substitutions), or correction of ion toxicity of the solution. In the absence of positive evidence that the nutrient solution contains all essential compounds in optimum amount before addition of the "stimulant", it is necessary to demonstrate that the "stimulant" is free of elements essential for the organism or capable of substitution for an essential element present in suboptimal amount, and that ion toxicity has not been corrected through addition of the poison.

The method of nutrient solution purification from heavy metals has proved the best means of obtaining accurate data in this field. The difficulty in obtaining compounds of sufficient and constant purity solely by means of recrystallization, or in different lots of commercially prepared chemicals, is well known and amply illustrated in the literature of this field. Data by Roberg (1928) affording a comparison between his own and others' results with the purification method show, however, exceptionally good agreement for physiological work of this character when it is taken into consideration that chemicals, glassware, etc. of entirely different origin were utilized.

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Some physiological effects of girdling northern hardwoods¹

HENRY I. BALDWIN

INTRODUCTION

In connection with a project for studying the effects of the release of the conifer understory by girdling the overtopping hardwoods, observations were made on the behavior of the hardwoods following girdling. The purpose of these observations was to determine the effectiveness of girdling in killing the decadent hardwoods, and how soon they would die and be removed from competition with the conifers.

A thorough review of the extensive literature on girdling will not be attempted here. T. Hartig (1858) was one of the first to observe the excess of food reserves above the ring over that below. He girdled young oaks in summer and on testing the wood for starch the following spring, found it had disappeared below the ring. Du Sablon (1905) showed that the bark must have some important function in food conduction. A large number of ringing experiments have been made, especially in horticulture, many give conflicting data on the effect of ringing in interrupting the translocation of foods. The later work of Curtis (1929) points to both upward and downward movement of carbohydrates, and to some extent inorganic nutrients in the phloem. Mason and Maskell (1928) in an exceptionally thorough investigation of translocation in the cotton plant found that ringing caused a blocking of downward transport and an accumulation of carbohydrates in the wood above the ring. The bulk of the present evidence indicates that the greater part of elaborated food material moves downward through the phloem. Many of the results of girdling experiments have been contradictory, however, and therefore the present data on the relative amounts of food reserves in girdled trees may be of interest. Furthermore, comparatively few observations have been made on the northern hardwoods, yellow birch (*Betula lutea* Michx.), beech (*Fagus grandifolia* Ehr.) and sugar maple (*Acer saccharum* Marsh.) and accompanying species. The work was carried on near Cupsuptic Lake in northwestern Maine, and in northern New Hampshire in 1925 and 1926. It should be borne in mind that these observations were incidental to the main project, and hence are very fragmentary.

¹ The results presented here are taken from reports of experiments on girdling hardwoods carried on from 1924-1932 for the Brown Company, Berlin, N. H., through whose courtesy the data is made public.

METHODS

1. *Girdling*. Trees were girdled by chopping rings around the bole at waist height. Three methods were used: (a) Notching, or chopping out a clean notch one to two inches into the sapwood, similar to the cut made when felling a tree. The notch was made completely around the tree. This method, while most certain of severing all phloem strands, and in addition, cutting into the xylem, demanded more skill, and was generally more expensive than others. (b) Peeling, or stripping the bark on a belt six inches to one foot wide around the tree. This was easiest to accomplish, and most certain of results when done in spring. (c) Hacking, or frilling the bark by downward cuts of the axe. This was cheapest, but found to be unreliable, since phloem strands would occasionally be left intact if the chops were not completely overlapping. Therefore it was soon superseded by double hacking, which has given the best satisfaction from all points of view. While trees were girdled at all seasons of the year for experimental purposes, the superiority of late winter from the standpoint of cheapness and organization of the work made other seasons out of the question. All observations here reported refer to trees girdled in February and March.

2. *Observations*. General notes were made of the condition of the trees whenever they could be visited. These were correlated with the data on the practical aspects of girdling, such as what species and sizes were most difficult to girdle. On sixteen one-acre permanent plots all trees were numbered, mapped and crown projections plotted. All trees were classified as to vigor and condition before girdling, and these observations compared with those made at intervals after girdling. General observations were also made on the larger areas of commercial girdling.

3. *Sampling*. One and two years after girdling blocks four inches high were sawed out of girdled and control trees one foot above the girdle and one foot below the girdle. These samples were taken during freezing weather, and placed in large mason jars for transport to the laboratory. There they were chipped, moisture content determined, and dextrose and total sugar content determinations made for each sample, using procedure recommended by the Association of Agricultural Chemists Methods of Analysis, 1925 (page 190).

RESULTS

The first growing season. The first observation was the drying of exposed sapwood, followed by the bleeding of sap from the cut trees with the advent of the maple sugar season. When the snow disappeared in May and warmer weather arrived fungus growths appeared on the sap. Birch trees were especially conspicuous because of a brilliant pink fungus. The

fungi consisted of a group of moulds, yeasts and bacteria, commonly known as slime flux. Toward June all bleeding and growth of fungi ceased, and the exposed sapwood felt dry to the touch.

Opening of the leaves and blossoming took place without any noticeable difference from control trees.

Autumnal coloration in girdled trees occurred from two to four weeks in advance of control areas. This was especially striking, the red and yellow

TABLE 1
Moisture content of girdled trees in per cent (based on oven-dry weight)

LOCATION OF SAMPLE	YELLOW BIRCH		SUGAR MAPLE		BEECH		AVERAGE OF ALL SPECIES AVERAGE				AVERAGE OF ALL METHODS AND LOCATION OF SAMPLE		
	NOTCHED	PEELED	NOTCHED	PEELED	NOTCHED	PEELED	NOTCHED	PEELED	OF BOTH	METHODS	YELLOW BIRCH	SUGAR MAPLE	BEECH
ONE YEAR AFTER GIRDLING													
Above cut	14.0	20.2	9.1	15.4	13.9	16.5	12.3	17.3	14.8	17.1	17.5	12.2	13.4
Below cut	20.1	16.7	13.9	15.4	8.9	10.8	14.3	14.3	15.3	18.4		14.6	
Controls ^a	16.8	16.8	17.6	17.6	13.6	13.6	16.0	16.0	16.0	16.8		17.6	13.6
TWO YEARS AFTER GIRDLING													
Above cut	18.3	14.5	8.4	12.8	17.8	14.7	14.8	14.0	14.4	16.4	16.0	10.6	12.6
Below cut	17.6	13.8	11.4	17.8	20.1	20.0	16.3	17.2	16.7	15.7		14.6	
Controls ^a	19.1	19.1	12.7	12.7	19.6	19.6	17.1	17.1	17.1	19.1		12.7	19.6

^a But one control sample was taken for each species.

foliage standing out conspicuously against the deep green of the forest outside the plots. By September some few trees had died, but most trees showed little effect of the girdling except premature coloration of the leaves. On analysis, it was found that about one-third of the trees denoted as "poor" in vigor before girdling had actually improved and been given a higher rating. Girdling may have so trapped reserve foods in the bole as to nourish dying branches better than before. Such effects must, of course, be temporary. Similar conditions have been observed after spring forest fires which girdled, but did not kill the trees outright.

Sprouting from the edge of the ring occurred in a few trees, chiefly beech.

No difference in the seed production could be ascertained, but no detailed measurements were made. All trees, whether girdled or not, bore profuse crops of seed.

The second growing season. Many trees showed marked weakening in vigor the second season. Leaf development was poorer; both the average leaf size was smaller, and many leaves withered and dropped soon after opening. Autumnal coloration again was premature. In some cases seeding was abundant. The more sensitive species such as black ash, black cherry, beech and paper birch were nearly all dead by the second season.

Relative sensitivity to girdling in relation to physical difficulty of girdling

operation. A relation was noted between the physical ease or difficulty of cutting into the tree and the promptness with which the trees died. This conclusion was based on a tally of trees on sample plots in September, 1925, one growing season after girdling, and on studies of the time re-

TABLE 2
Moisture content of girdled trees in per cent of control trees

LOCATION OF SAMPLE	YELLOW BIRCH		SUGAR MAPLE		BEECH		AVERAGE OF ALL SPECIES				AVERAGE OF BOTH METHODS		
	NOTCHED	PEELED	NOTCHED	PEELED	NOTCHED	PEELED	NOTCHED	PEELED	BOTH		YELLOW BIRCH	SUGAR MAPLE	BEECH
ONE YEAR AFTER GIRDLING													
Above cut	83.3	120.5	51.5	87.5	101.0	120.5	78.6	109.5	96.0	101.9	69.5	110.2	
Below cut	119.0	99.1	79.0	87.5	65.3	79.1	87.7	88.5	88.1	109.0	83.2	72.2	91.4 ^a
TWO YEARS AFTER GIRDLING													
Above cut	96.0	75.5	66.1	100.2	91.0	75.0	84.3	83.5	83.9	85.7	83.1	83.0	
Below cut	92.5	72.1	89.7	140.0	102.5	102.1	94.9	104.7	99.8	82.3	114.8	102.3	92.6 ^a

^a Average of above and below cut.

quired to girdle each tree. The softer woods, comprising the species more intolerant of shade succumbed first. These were the species which furnished the easiest chopping. Many of these species are relatively short-lived, and

TABLE 3
Moisture content of girdled trees above the ring in per cent of content below the ring

	YELLOW BIRCH		SUGAR MAPLE		BEECH		AVERAGE OF ALL SPECIES				AVERAGE OF BOTH METHODS		
	NOTCHED	PEELED	NOTCHED	PEELED	NOTCHED	PEELED	NOTCHED	PEELED	BOTH		YELLOW BIRCH	SUGAR MAPLE	BEECH
One year after girdling	69.5	121.0	65.2	100.0	156.1	152.5	96.9	124.5	110.7		95.2	82.6	154.3
Two years after girdling	104.0	105.0	73.8	72.0	88.4	73.5	88.7	83.5	86.1		104.5	72.9	80.9

are not as severe competitors of the desirable conifers as the more tolerant hardwoods.

Moisture content of girdled trees. The results of analyses of samples taken from random trees one and two years after girdling are given in Tables I-III. Girdled trees did not differ markedly from control trees in moisture content during the first two winters following girdling. In fact there was generally greater range between different control trees different years than between controls and girdled trees (Table I). Obviously many more trees would have to be sampled to obtain conclusive data. The present material suggests a lower water content on the average for girdled trees. During the first year there was usually higher water content above

the ring than below, while the second year this condition was reversed. Peeled trees averaged a higher water content than notched trees, reflecting the greater interruption of xylem in notched trees.

Sugar content. In Table IV are given sugar contents in both invert

TABLE 4
Analyses of girdled trees

LOCATION OF SAMPLE	WATER CONTENT PER CENT		WEIGHT OF SOLUBLE CARBOHYDRATES IN MG. PER 20 GM. DRY WOOD			
	1-yr.	2-yr.	INVERT SUGAR AND SUCROSE ^a		DEXTRASE ^a	
			1-yr.	2-yr.	1-yr.	2-yr.
			YELLOW BIRCH NOTCHED			
Above ring	14.0	18.3	230.4	22.1	230.0	23.6
Below ring	20.1	17.6	0.3	2.3	0.3	4.7
			YELLOW BIRCH PEELED			
Above ring	20.2	14.5	184.8	1.1	196.0	3.0
Below ring	16.7	13.8	26.8	7.0	35.6	9.2
			YELLOW BIRCH CONTROL			
	16.8	19.1	61.6	48.2	78.4	48.3
			SUGAR MAPLE NOTCHED			
Above ring	9.1	8.4	216.8	8.1	227.2	10.4
Below ring	13.9	11.4	6.8	0.8	16.4	2.0
			SUGAR MAPLE PEELED			
Above ring	15.4	12.8	231.2	76.9	240.0	76.0
Below ring	15.4	17.8	6.4	27.3	16.0	28.5
			SUGAR MAPLE CONTROL			
	17.6	12.7	104.8	86.6	120.0	85.3
			BEECH NOTCHED			
Above ring	13.9	17.8	0.0	192.3	0.0	187.8
Below ring	8.9	20.1	4.3	10.7	6.6	12.6
			BEECH PEELED			
Above ring	16.5	14.7	20.0	131.2	29.0	128.3
Below ring	10.8	20.0	54.8	5.7	62.0	7.9
			BEECH CONTROL			
	13.6	19.6	58.0	43.9	65.2	44.4

^a Computed from Munson and Walker's Tables.

sugar and dextrose, as computed from Munson and Walker's tables. It will be observed that the contents in each class of sugars follow one another very closely, and any conclusions for one group apply equally well for the other. Accordingly figures for dextrose only were used in computing Table V which shows more clearly the comparison between girdled trees and normal trees.

DISCUSSION

The water content of girdled trees was less, but showed little difference from ungirdled trees, and the difference was less the second year than the first. The fact that water contents showed as much difference as they did suggests that the girdling even by peeling, with drying out of the surface of the sapwood caused an interruption of the sap stream, either by clogging with air, or actually killing part of the water conductive tissue. The part played by this influence on water conduction may be of great importance in causing the death of the tree than would be at first supposed.

TABLE 5
Dextrose content of girdled trees in per cent of control trees

LOCATION OF SAMPLE	YELLOW BIRCH		SUGAR MAPLE		BEECH	
	NOTCHED	PEELED	NOTCHED	PEELED	NOTCHED	PEELED
ONE YEAR AFTER GIRDLING (1926)						
Above cut	293.2	250.0	189.3	200.0	0.0	44.4
Below cut	0.4	45.4	13.6	13.3	10.1	95.0
Controls	100.0	100.0	100.0	100.0	100.0	100.0
TWO YEARS AFTER GIRDLING (1927)						
Above cut	48.8	6.2	12.2	89.1	423.0	288.9
Below cut	9.8	19.0	2.3	33.3	28.4	17.8
Controls	100.0	100.0	100.0	100.0	100.0	100.0

Experiments in dyeing living trees showed that in virgin forest hardwoods a very narrow shell of sapwood was used in sap conduction.

In both seasons water contents were generally highest where there was least sugar, and where evidences of decay were most prominent. From the tables it will be noted that a conspicuously high water content above the cut occurred in beech one year after girdling, when a very low sugar content was also observed. Therefore decay is indicated as the cause of high water content. The higher water content above the ring than below is at variance with the reputed effect of girdling on cypress and teak, as well as other tropical trees, and suggests that girdling would be of no use in increasing the buoyancy of hardwood for river-driving. This agrees with the findings of Curtis.

Beech was an exception and apparently behaves differently than yellow birch and maple both when notched and peeled. The sugar contents were almost the exact opposite of the other species, low the first year and extremely high the second; higher below the cut the first year, and above the cut the second. These first year's results strongly suggest the activity of root suckers in keeping the old trunk supplied with carbohydrates. Presence of decay in the samples may have had some influence on the sugar

analyses. Differences in the root system, and silvical characteristics are probably also responsible.

The water content of beech compared to the other species may be due to the restriction of actively conducting xylem in beech to a narrower zone near the bark, than is the case with yellow birch and sugar maple. This is not fully proven, but experiments in injecting dyes into living trees at Dummer, New Hampshire in 1925 point to this as a possible explanation. This might also explain the retention of a large amount of water the first year after girdling, had transpiration been cut off; but it was observed that all beeches from which samples were sawed were in full leaf during the first growing season. Beech leaves are, however, heavily cutinized, and yet were observed to be partly wilted in late summer, which would have tended to close the stomata and reduce transpiration. It is possible that beech was so severely affected by girdling that forces tending to cause sap rise were impaired. In other species transpiration may have tended to pump dry the outer layers of the sapwood.

Sugar contents follow opposite trends from water contents. Yellow birch and sugar maple showed huge accumulations of sugar above the cut as compared to parts of the same tree below the ring, or to control trees. There was usually 20 to nearly 300 times as much above the cut as below and 2 to 3 times as much above the cut as in control trees of the same species. This agrees well with what other investigators have found, and confirms the usually accepted theory that the bulk of the carbohydrates move down in the phloem. The excess of sugars above the cut was quickly discovered by porcupines, who gnawed the bark of large numbers of trees, above the cut exclusively. Beech again showed quite contrary results from the other trees tested. In the case of notched beech, no sugar whatever was found above the ring the first year. Possibly the sugar above the ring was withdrawn to a large extent for starting growth; when the leaves opened and shortly thereafter, water conduction may have been so restricted that stomata were kept partially closed, and little carbon was assimilated. Stored food was depleted, and little or none laid up for the next season. The second season most of the beeches sent up small sprouts from the bottom of the cut. This, however, does not explain the higher sugar content above the ring observed in the second winter.

The results do not show conclusively whether notching or peeling is more effective in killing the tree, but there is little doubt that notching causes the more severe injury. It is curious to note that in sugar maple the sugar contents below the girdle the first year should have been almost identical for both methods, while in other cases notching was more effective in reducing the content below the ring as compared to peeling. The

second year this is shown also in sugar maple. This comparison between peeling and notching suggests that some carbohydrates may possibly be carried down in the outer layers of xylem.

The sugar contents reflect the ability of the trees to remain alive after girdling, and agree with the observed relative vitality of different species. Beech was thus rated considerably lower than the other two species in resistance to girdling. Trees with least sugar are apparently most sensitive to girdling. This is not in complete agreement with the accepted grouping of trees by their reserve foods; beech and maple are considered predominantly starch trees while birch is predominantly a fat-reserve tree. The indications were the first year that beech would succumb promptly, but the presence of root suckers may account in part for observed exceptions.

The premature coloration of the leaves might possibly have had some connection with a damming up of sugar in the upper part of the bole, which would have led to excess development of anthocyanin pigments, and coupled with reduced sap flow, caused premature development of the abscission layer.

SUMMARY

Observations are reported on the condition of girdled hardwoods during the first two years after girdling. Girdling was done in winter. These were supplemented by analyses of samples cut from trees one and two years following girdling.

1. Three to four years were required for girdled hardwoods to die completely. Some species died the first year. About a third of the trees which seemed low in vigor before girdling seemed to have been temporarily benefited the first summer.

2. Short-lived, intolerant and soft-textured species succumbed more rapidly than long-lived, tolerant, and dense-wooded species.

3. The most noticeable change in the trees the first season was premature autumnal coloration.

4. Girdled trees had about the same moisture contents as normal trees, usually slightly less.

5. Moisture content above the ring was about 10 per cent higher the first year, and 10 per cent lower the second year after girdling, as compared to the content below the ring.

6. A relatively high moisture content was found where the sugar content was low, and vice versa.

7. Invert sugars and sucrose showed the same general distribution as dextrose.

8. The first season after girdling abnormally large per cents of sugar

were trapped above the girdle. There was 20 to 300 times as much sugar above the cut as below, and 2 to 3 times as much as in normal trees.

9. Notching was more effective than peeling in causing carbohydrates to be concentrated above the cut. Interruption of the sap stream from carrying reserves up from the roots may also account for low sugar content below the cut.

10. Two years after girdling, sugar reserves were much depleted and were less even above the cut than in normal trees.

11. Beech differed from yellow birch and sugar maple in the relative distribution of sugar and water, and the results were generally erratic for this species.

12. The amount and distribution of carbohydrate reserves seems to be correlated with the resistance of the various species to injury by girdling. Trees which showed visible injury in foliage, etc., most promptly, also showed depletion of food reserves, and abnormality of water content.

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Field and herbarium studies. II.

LOUIS WILLIAMS

This paper is the second in a series based upon studies made at the Rocky Mountain Herbarium. The new names in *Lepidium* are proposed as a result of studies made in revising the genus for a new Flora now in preparation.

Lepidium Nelsonii sp. nov. Bienne aut perenne caducum; caulibus compluribus ex corona, 2–4 dm. altis, ramosissimis ex medio sursum, denso pubescentibus crinibus erectis; foliis oblongis vel linearibus, serratis, lacinatis aut pinnatifidis, utrinque pubescentibus; inflorescentia densa, in fructu multo elongata; petalis nullis aut perparvis; sepalis perparvis, albis; staminibus 2; stigmatibus sessilibus; silicula pubescente, circa 3 mm. longa; embryo notorrhizo.

Ascending biennial or short-lived perennial. Stems several from the crown, 2–4 dm. high, much branched from the middle upward, densely pubescent with erect hairs; leaves oblong to linear, serrate, lacinate or pinnately parted, moderately harsh pubescent on both surfaces, the cauline sessile; inflorescence dense, much elongated in fruit; petals absent or every minute; sepals minute, about 0.5 mm. long, white; stamens 2; stigma sessile in the notch of the silique; pubescent, orbicular, about 3 mm. long and a little less wide; pedicels divaricate, about 3 mm. long, cylindric; cotyledons incumbent.

Type, *Aven Nelson 11,417*, collected May 25, 1931, in low hills around Frijoles, New Mexico. *L. Nelsonii* is the most pubescent of the known southwestern species, from *L. hirsutum* Rydb. it can be easily separated by the pubescent siliques, from the forms of *L. virginicum* L. by the cotyledons.

Lepidium tenellum sp. nov. Perenne adscendens; caule 2–4 dm. longo, gracili, puberulo aut infra glabro; foliis basalibus oblongis, 1–3 cm. longis, incisis, petiolo gracili; foliis caulinis superioribus oblanceolatis, integris, inferioribus saepe incisis; inflorescentia ante anthesin densa, in fructu valde elongata, 4–7 cm. longa; pedicello reflexo, 5–6 mm. longo; calyce pubescente, ovato, albo aut saepe ad marginem purpureo, 1 mm. longo; corolla alba, orbiculari, 2 mm. longa; staminibus 6; silicula glabra, 3 mm. longa, 2.5 mm. lata, suborbiculari, margine alata; incisura fere obsoleta; stylo circa 1 mm. longo, valde exserto; stigmatibus subcapitato.

Ascending from large perennial clumps. The stems 2–4 dm. long, minutely puberulent or the lower part glabrous, very slender; basal leaves oblong, deeply incised, about 3 cm. long, the petiole slender, longer than the blade; upper cauline leaves oblanceolate, entire, almost sessile, the lower cauline leaves often incised or entire, 1–3 cm. long, petiolate, the petiole almost as long as the blade, often winged; inflorescence crowded before anthesis, much elongated in fruit; raceme lax, 4–7 cm. long; pedicels reflexed, spreading, 5–6

mm. long; sepals sparingly pubescent, ovate, white or often purple margined, 1 mm. long; petals white, orbicular, 2 mm. long, clawed, the claw exceeding the calyx; stamens 6; silique 3 mm. long, 2.5 mm. wide, glabrous, suborbicular, wing-margined; the notch almost obsolete; style about 1 mm. long, much exerted; stigma subcapitate.

Type, *Edwin B. Payson 1033*, July 8, 1917, in "Large perennial clumps in rock crevices near the R.R., Black Canyon of Gunnison, Colorado." Easily distinguished from the other Rocky Mountain *Lepidium*s by the very slender ascending stems and the lax, few fruited racemes. Distributed as *L. scopulorum*? Jones.

Lepidium tortum sp. nov. Perenne ex rhizomate magno; caulibus adscendentibus aut rectis, 20–40 ex corona, 1–3 dm. altis, puberulis, in aetate glabrescentibus, ramosissimis, ad nodos angulosis; foliis caulinis linearibus, integris, breviter acuminatis, 2–6 cm. longis; inflorescentia densa, in fructu usque ad 8 cm. longa elongata; sepalis albis, oblongis, 2 mm. longis; pedicellis 5–7 mm. longis, cylindratis, pubescentibus, divaricatis; silicula glabra, ovata, circa 3 mm. longa, 2 mm. lata; incisura evidente; stylo longo exserto, 1 mm. longo; stigmatе globoso; seminibus 1.5 mm. longis; embryo notorrhizo.

Perennial from a large root-stock. Stems ascending or erect, 20–40 from the crown, 1–3 dm. tall, puberulent, becoming glabrous in age, much branched the whole length, angled at the nodes; cauline leaves linear, entire, short-acuminate, 2–6 cm. long; basal leaves not seen; inflorescence dense, racemes elongated in fruit, up to 8 cm. long; pedicels 5–7 mm. long, cylindric, pubescent or in age glabrous, divaricate; sepals white, oblong, 2 mm. long; petals white, ovate, 3 mm. long, claw 2 mm. long; silique glabrous, about 3 mm. long, 2 mm. wide, ovate, notch apparent; style long exerted, 1 mm. long; stigma globose; seed 1.5 mm. long, ovate, hyaline winged only near point of attachment, developing a gelatinous sheath when boiled; cotyledons incumbent.

Type, *L. N. Goodding 2281*, May 4, 1905, on ridges and slopes near Las Vegas, Nevada. Other collections are: *A. O. Garrett 5976*, July 23, 1931, west of Hawksville, Wayne C., Utah and *5980*, Wayne Co., Utah, *5930* Emery Co., Utah. Related to *L. alyssoides* Gray, it may be readily separated from that species by the long exerted style and the much angled stems, giving a ragged appearance to the plant.

Horkelia sabulosa (M. E. Jones) comb. nov.

Potentilla sabulosa M. E. Jones. Proc. Calif. Acad. II. 5: 680. 1895.

Ivesia sabulosa M. E. Jones. l.c. As a synonym.

Comarella sabulosa Rybd. Mem. Dep. Columbia Univ. 2: 157. 1898.

Potentilla albiflora sp. nov. Perennis humilis, 10–15 cm. alta; radice magna radiculis multis fibrosis; corona foliosa, ramis compluribus ex quaque corona; foliis trilaminatis, 5–15 mm. longis, 4–10 mm. latis, supra medium

crenatis breviter piloso-pubescentibus; foliis caulinis variis; petiolis foliorum in basium gracilibus, 6–12 cm. longis, breviter piloso-pubescentibus; inflorescentia cymosa pauciflora; lobis calycis acutis, 3 mm. longis; corolla alba, lateobovata, 3 mm. longa; bracteis lanceolatis, 2 mm. longis, glanduloso-pubescentibus; acheniis circa 20 in quoque capite conico, glabratiss.

A low alpine (?) perennial, 10–15 cm. tall. Roots large, bearing many small fibrous roots; crown leafy, leaves reaching almost to the inflorescence by a slender petiole, several flowering branches from each crown; leaves trifoliate, the middle leaflet 10–20 mm. long, 6–14 mm. wide, the lateral leaflets smaller, sessile or very short petiolulate, crenate above the middle by ovate lobes about 1 mm. long, short pilose-pubescent on both sides, that of the upper surface thicker and somewhat appressed; stem leaves various, some small and entire, 3–6 mm. long, 1 mm. wide, sessile, some short petiolate, trifoliate, the leaflets oblong and entire or tridentate at the apex, 8–12 mm. long, 4–6 mm. wide; petioles of the basal leaves slender, striate, 6–12 cm. long, moderately short pilose pubescent; inflorescence a few-flowered cyme; calyx lobes acute, gradually widening to the base, pilose-pubescent, 3 mm. long; petals white, broadly obovate, 3 mm. long, barely equaling the calyx; bracts lanceolate, 2 mm. long, pubescent and often ciliate with short glandular hairs; achenes about 20 in each cone-shaped head, about 1 mm. long, glabrate.

Type, *L. N. Goodding 1045*, near Fort Grant, Arizona on rocky, alpine slopes, June 15, 1912. Not closely related to any species known to the writer; in aspect resembling *P. maculata* Pourret, from which it differs in having only 3 leaflets which are not so deeply toothed and a white corolla, as well as a different geographical distribution.

Potentilla rubida sp. nov. Perennis erecta, gracilis, pauciflora, 5–8 dm. alta, ex rhizomate crasso, glanduloso—pubescens; foliis basalibus 5–6 digitatis; petiolis 1–3 dm. longis; foliolis sessilibus, oblongis aut oblongo-oblanceolatis, 2–7 cm. longis, serratis praeter mediam partem; stipulis magnis, 2–4 cm. longis, 5 mm. latis; foliis caulinis similibus sed minoribus; calyce 7 mm. longo, lanceolato-acuminato, pubescente et glanduloso aut glabro; corolla rubida, petalis obcordatis, 7–10 mm. longis, 5–8 mm. latis; staminibus circa 20; pistillis numerosis.

Erect, slender, few-flowered perennial 5–8 dm. tall, from a thick root-stock. Usually only one stem from each root, glandular-pubescent, more so above than below, scattering hairs near the base; basal leaves 5–6 digitate; petioles 1–3 dm. long, slender, glandular-pubescent; leaflets sessile, oblong to oblong-oblanceolate, 2–7 cm. long, sharply serrate for half or three-fourths of their length, minutely glandular on both surfaces, the veins on the lower surface prominent, pubescent with long hairs; stipules large, 2–4 cm. long, 5 mm. wide, sheathing, adnate to the petiole, having a linear-acuminate lobe on each side 6–15 mm. long, pilose; stem leaves similar but smaller, the petioles

short or the upper almost sessile, the stipules broad and often with one or more acute lobes; calyx lobes lanceolate-acuminate, about 7 mm. long, softly pubescent and minutely glandular or almost glabrous; bracts equaling the calyx, linear-acuminate; corolla dark purple, the petals obcordate, 7–10 mm. long, 5–8 mm. wide, the petals in the bud pustulate, the pustules containing a dark red liquid; stamens about 20, inserted on the reddish disk; pistils numerous.

Type, *C. H. T. Townsend* and *C. M. Barber* 49, in part, July 4, 1899, collected near Colonia Garcia, Chihuahua, Mexico. Distributed as *P. Thurberi* Gray, to which it is most nearly related; it may be distinguished by the oblong stipules which are not toothed but have two linear, lateral lobes, the leaflets are oblong to oblong-spatulate not obovate, the sepals are lanceolate-acuminate not triangular-acute.

Parosela Paysoniae sp. nov. Perennis humilis lignea, 2–3 dm. alta; caulibus multis ex radice, ramosis, denso sericeis ex medio sursum, glandulis nigris aut flavis punctatis; foliis 1–3 cm. longis, imparipinnatis 3–6-jugis; foliolis 5–8 mm. longis, 2–3 mm. latis, glanduloso-punctatis, dense sericeis; inflorescentia conferta, oblonga, 2–3 cm. longa, 1 cm. lata; staminibus 10, filamentis monadelphis; calyce circa 5 mm. longo, sericeo, nonglanduloso; corolla purpurea, circa 6 mm. longa; legumine sericeo, punctato-glanduloso; semine uno; stylo 6–7 mm. longo, ad basin pubescente, sursum glabro.

Low woody perennial, 2–3 dm. tall. Many stems from the base, branching almost their entire length, glabrous and roughened near the base, becoming densely sericeous from the middle upward, dotted with black or yellow glands the full length; leaves 1–3 cm. long, odd pinnate with 3–6 pairs of leaflets; leaflets oblong-oblancoate, densely glandular-punctate, sericeous on both surfaces, 5–8 mm. long, 2–3 mm. wide; inflorescence crowded, oblong, 2–3 cm. long, 1 cm. wide; stamens 10, monadelphous; filaments united two-thirds their length; calyx sericeous, about 5 mm. long, the subulate teeth 2 mm. of this, not glandular, sessile on the rachis; corolla purple, the keel united half its length, saccate, long clawed; wings and banner not seen; pods sericeous, at least when immature, glandular-punctate, one seeded; style 6–7 mm. long, pubescent at the base, glabrous upward.

Lois B. Payson 37, September 27, 1931, rocky slopes near Carlsbad Caverns, New Mexico. Related to *P. lanata* (Spreng.) Britt., from which it differs in having a more dense and broader inflorescence, woody stems and the absence of glands on the calyx.

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Inheritance of cotyledonary characters in *Cucurbita Pepo*

GARDIS B. THAYER

(WITH TEXT-FIGURE)

Comparatively little work has been done on the inheritance of cotyledonary characters. Mendel (1865) found that the yellow color of the cotyledon in peas was dominant over green and that there was a difference of a single factor between these traits. Hill (1925) reported that in *Digitalis* "the F_1 hybrids between species with differing types of cotyledons show strong matroclinous tendencies," but that "matrocliny is not complete, since the influence of the pollen parent is seen in the ratio of the width to the length of cotyledons." He explained this matrocliny as due to the relation between the maternal seed coats and the developing embryo. Michaelis (1931) confirmed Hill's results. Focke (1881) observed that in *Nymphaea* species hybrids the cotyledons resemble those of the female parent. The inheritance of cotyledonary size is complicated somewhat by heterosis. Thus Wingard (1927) found that bean seeds which arose through cross pollination were larger and had larger cotyledons than those which arose by self pollination. The results of Rosa (1926), however, indicate that in melons there is no significant difference in size between seeds arising from the two types of pollination. It is reasonable to expect that characters of the embryo which depend more or less directly upon the characters of the seed in which the embryo is produced should show matroclinous inheritance.

MATERIAL AND METHODS

The purpose of the present work was to study the inheritance of certain cotyledonary characters in *Cucurbita Pepo*. Seeds of various pure lines of this species and of F_1 and F_2 progenies from crosses between them were available from the cultures of Professor Sinnott. Soaked seeds were germinated in an incubator until the hypocotyl appeared and were then planted in flats. When the first foliage leaf was about one centimeter broad the seedlings were harvested and preserved in alcohol. After the cotyledons had become bleached they were detached and placed between thin glass plates which were inserted in the slide-holder of a projection lantern. The outlines and venation of the cotyledons were now projected and traced on drawing paper at three times their actual size. Certain measurements were taken from the cotyledons themselves and others from these projected drawings. In the pure lines and F_1 , ten seedlings of each were studied, but at least twenty for each F_2 pedigree. For size measurements and shape

TABLE 1

Inheritance of cotyledonary shape index (length/width)

PURE LINES	MEAN	COEF. VAR.
C	1.35±.017	5.7±.87%
FSC	1.59±.014	4.1±.62
M	1.41±.024	8.1±1.10
MAR	1.78±.032	8.3±1.13
P	1.66±.020	5.6±.85
5	1.58±.024	6.9±1.05
10	1.49±.015	5.3±.81
22	1.21±.017	6.6±1.01
46	1.26±.012	4.2±.64
103	1.57±.013	3.8±.58
125	1.50±.026	8.0±1.22
391	1.25±.019	7.1±1.08
438	1.35±.024	8.1±1.10
741	1.46±.018	5.6±.85
BOT	1.70±.033	8.8±1.19
F₁ and F₂ Pedigrees		
F ₁ 103 x MAR	1.47±.013	4.1±.62
F ₂ 103 x MAR	1.62±.012	6.1±.52
F ₁ 125 x 22	1.45±.017	5.3±.81
F ₂ 125 x 22	1.47±.074	4.6±.39
F ₁ M x C	1.51±.014	4.3±.54
F ₂ M x C	1.50±.011	5.2±.53
F₁ Progenies		
103 x 22	1.66±.018	5.3±.81
22 x 103	1.39±.014	4.7±.72
438 x 5	1.36±.016	5.4±.82
5 x 438	1.45±.019	6.4±.98
103 x C	1.43±.017	5.3±.81
C x 103	1.43±.023	7.4±1.13
10 x 391	1.47±.009	2.7±.41
391 x 438	1.34±.012	3.9±.59
46 x 438	1.36±.014	4.8±.73
391 x FSC	1.36±.015	5.1±.78
M x FSC	1.41±.017	5.6±.85
C x P	1.53±.013	3.8±.58
P x 103	1.64±.012	3.2±.49
741 x P	1.43±.009	2.8±.42
5 x P	1.57±.013	3.7±.56
F₂ Progenies		
BOT x 103	1.66±.013	5.0±.47
103 x M	1.57±.019	8.4±.85
BOT x 10	1.56±.013	7.1±.59
BOT x 22	1.51±.013	7.4±.61
BOT x 46	1.56±.012	6.5±.56
BOT x 125	1.52±.017	6.8±.74

indices the two cotyledons from a seedling were averaged but for vein characters only one was used. Seed measurements (length and width) were also made on ten typical seeds from a plant. For cotyledons, the length and width of the blade were determined, as well as the maximum width of the two main vein islets, the distance between the points of origin of the first two branches of the midrib, and the angle between these branches. In addition to these quantitative characters certain qualitative differences were studied.

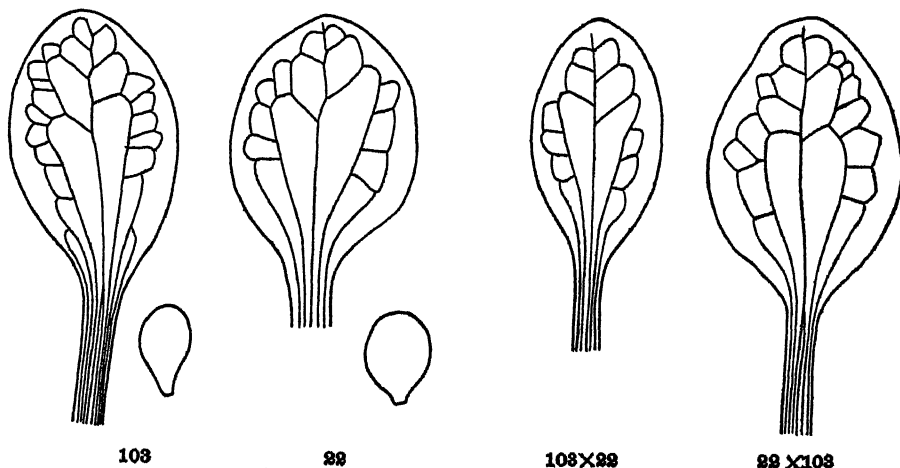


Fig. 1. At left, cotyledons of pure lines 103 and 22, with outlines of seeds of these lines, showing relation of seed shape to cotyledon shape. At right, cotyledons of the two reciprocal crosses between these lines, showing marked matrocliny in cotyledon shape inheritance.

RESULTS

Size of cotyledons. This character is complicated by the marked influence of environmental conditions as well as by heterosis. The results of the present study, however, confirm the conclusions of previous workers that cotyledonary size is controlled in large part by the size of the seed in which the embryo is produced, and is thus matroclinous in its inheritance.

Shape of cotyledons. Table 1 presents data as to the mean and coefficient of variability for cotyledonary shape index (length of blade divided by width) in the pure lines and the various F_1 and F_2 progenies from crosses between them. The first group includes the pure lines, and marked differences in cotyledonary shape are evident among them. In the second group are three F_1 progenies with their corresponding F_2 offspring. The third group contains F_1 pedigrees from which no F_2 had been grown. In the fourth group are a number of F_2 pedigrees for which the F_1 was not available.

An examination of this table discloses a number of general facts with regard to shape inheritance. First, the mean index of an F_1 is in most cases much closer to that of its seed parent than to that of its pollen parent. Second, the variability of the F_2 generations is not strikingly higher than that of the F_1 and on the average is about the same as that of the pure lines. Third, the mean of the F_2 bears no necessary relation to that of the F_1 and is usually intermediate between the means of the two pure lines which were crossed.

TABLE 2
Comparison of shape indices of seeds and cotyledons

PURE LINES	SEED SHAPE INDEX	COTYLEDON SHAPE INDEX	CROSSES	F_1 SEED SHAPE INDEX	F_2 COTYLEDON SHAPE INDEX
BOT	1.75	1.70	103 x MAR	1.71	1.62
C	1.48	1.35	MAR x 103	1.77	1.71
CRK	1.80	1.54	BOT x 103	1.77	1.66
FLIW	1.75	1.68	103 x BOT	1.70	1.41
M	1.63	1.41	BOT x 10	1.76	1.56
MAR	1.75	1.78	10 x BOT	1.76	1.56
9	1.62	1.49	BOT x 46	1.68	1.56
10	1.70	1.49	46 x BOT	1.62	1.35
13	1.48	1.22	103 x M	1.62	1.57
22	1.32	1.21	125 x 22	1.74	1.47
43	1.68	1.51	391 x 741	1.54	1.38
46	1.67	1.26	M x C	1.57	1.50
90	1.60	1.50	BOT x 22	1.58	1.51
103	1.67	1.57	BOT x 43	1.71	1.42
105	1.53	1.54	BOT x 543	1.69	1.40
125	1.84	1.50	BOT x C	1.49	1.49
391	1.60	1.25	BOT x CRK	1.75	1.66
543	1.51	1.30	BOT x FLIW	1.93	1.74
741	1.46	1.46	9 x BOT	1.64	1.34

These facts can be readily explained if it is true that seed shape (a maternal character) directly determines cotyledon shape. The resemblance of the F_1 cotyledons to those of their female parents would thus be due to the fact that both were developed in seeds of the same shape. Neither segregation nor increase in variability would be expected in the cotyledons of the F_2 since these are all grown in F_1 seeds, which are essentially uniform in shape. Finally, there would be no necessary relation between the mean of the F_1 and the mean of the F_2 , since the former would resemble the maternal pure line and the latter would be determined by the seed shape of the F_1 plants, which might be quite different from that of either parent.

To determine whether any direct relation exists between seed shape and cotyledon shape, the average shape indices for seeds and for the coty-

ledons of seedlings grown from these seeds were compared. The results in various pure lines and in hybrids between them are presented in Table 2. Although the seed index is higher than its corresponding cotyledonary index, the two indices tend to vary together. The coefficient of correlation between seed shape index and cotyledon shape index in the pure lines is found to be $+.52 \pm .036$, and between F_1 seeds and F_2 cotyledons, $+.64 \pm .083$. These facts indicate that seed shape does determine cotyledon shape to a considerable degree.

Although cotyledon shape seems thus to be influenced by seed shape and therefore to exhibit a certain degree of maternal inheritance, matrocliny is by no means complete. It is evident from Table 1 that certain pure lines behave differently from others in their effect on the F_1 . In the cross of line 438 pollinated by line 5, for example, the F_1 cotyledons closely resemble those of 438, but in the reciprocal cross, 5×438 , they are intermediate in character. Similarly, 103×22 resembles 103 whereas 22×103 is intermediate (fig. 1). In these crosses, lines 438 and 103 seem to have some effect on cotyledon shape when used as pollen parents but C and 5 do not. That this relationship does not always hold, however, is shown by the reciprocal crosses involving C and 103, in which the F_1 is intermediate in both cases. Line 438 seems to exert a considerable influence on cotyledon shape wherever it is used as a pollen parent.

Further evidence that there is a certain degree of normal biparental inheritance in cotyledonary shape is shown by the fact that the variability of the F_2 populations, while not at all high, is substantially higher than that of the F_1 , the average of the former being 6.3% and of the latter 4.6%. To be sure, the average variability of the pure lines is fully as high as that of the F_2 , but this is probably due to the lack of vigor which characterizes most pure lines and which results in the production of a good many small and defective seedlings, the presence of which in a population increases its variability.

We may conclude, therefore, that there is some indication of segregation here and that cotyledon shape, although markedly matroclinous in its inheritance, is not wholly so.

Vein characters. Between certain of the lines there are observable a number of slight but definite differences in cotyledonary vein pattern. The ratio of the width of the two main lateral islets to that of the cotyledonary blade, the distance apart in origin of the two main lateral veins arising from the midrib, and the angles which these make with the midrib were studied in certain pedigrees. In some crosses the vein pattern of the maternal parent would appear in the F_1 and in others that of the pollen parent.

Although critical evidence from reciprocal crosses was unfortunately not available in pedigrees where the greatest pattern differences occurred, it seems unlikely that these traits would show any evidence of matrocliny. This conclusion is strengthened by the fact that for these three characters the variability of the F_2 is consistently though not always significantly higher than that of the F_1 .

Other traits. In line 43, out of 95 seedlings studied, 52 showed a tendency toward the fusion of the two cotyledons, either partial or complete. Only one cross involving line 43 was studied and here it happened to be used as the pollen parent. A considerable number of cases of fusion were observed in the F_1 and F_2 , suggesting that this trait is inherited and can be transmitted through the pollen.

Certain differences in cotyledonary surface were also observed among the pure lines. A roughened or pebbly surface, caused by a swelling of the tissue between the veins, characterizes line 103. A lace-like pattern, due to the poor development of chlorophyll just above the veins, is found in line C. Both these traits behave as almost complete dominants, whether they enter a cross from the male or the female parent, and appear in a considerable portion of the F_2 , although their segregation there is not sharp.

SUMMARY

The inheritance of various cotyledonary characters was studied in crosses between pure lines of *Cucurbita Pepo*.

Size of cotyledons is markedly influenced by seed size and thus tends to show a high degree of matrocliny.

Cotyledonary shape (ratio of length to width) is also determined to some degree by the seed parent through the influence of seed shape upon the developing embryo, but the pollen parent exerts a considerable effect in many cases and there is evidence here of a certain amount of normal biparental inheritance.

Characters of vein pattern and cotyledonary surface seem not to be maternally determined but to display ordinary inheritance.

The character of the cotyledons in this species is thus the result of a balance between the growth tendencies implanted in the embryo by its own genetic constitution, and the limitations imposed by the character of the seed in which this growth must take place.

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1928-1933

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The cytology of the abscission zone in *Mercurialis annua*

CECIL YAMPOLSKY

(WITH FIGURES 1-8)

At the height of its vigor in mid-summer, hundreds of insects daily visit the male plants of *Mercurialis annua*. They are attracted by the characteristic odor given off by the many nectaries distributed over the inflorescences and also by the large amount of pollen that is produced. The flowers are short-lived. After dehiscence the anther sacs shrivel up and change from a yellow green color to a deep indigo. Such ephemeral and inconspicuous male flowers produced singly on female plants and which ultimately lead to the production of seed have in the past created the false assumption of parthenogenesis in *Mercurialis annua*.

When plants are hardly more than a month old their sex can be identified and from then on they continue to produce an increasingly large number of flowers. Flower production ceases only when the plant dies. During its life span a male plant produces and then discards tens of thousands of flowers. Whenever a flower is thrown off a truncated pedicel is left behind. The cells of the pedicel remain alive as long as the inflorescence, which is an interrupted spike, remains attached to the plant. When a flower is discarded a scar is left behind and that means a place on the plant through which liquids could escape if no provisions were made to prevent such an occurrence.

In studying the cytology of the male, female and hermaphrodite flowers of *Mercurialis annua* I was impressed with the well defined abscission zones in the region somewhat below the receptacle. R. von Wettstein (1916) found a correlation between the abscission zone in the male flower of *Mercurialis annua* and wind pollination. He was of the opinion that this plant had evolved an adaptive mechanism to further wind pollination. He observed that just before dehiscence, the perianth leaves become recurved so that their tips press against the pedicel. Prior to that the separation of the flower from its subtending structure had started. The separation begins at the periphery of the pedicel and proceeds inward towards the central vascular strand. Curvature of the perianth leaves is brought about by the expansion of specialized cells (*Schwellgewebe*) found at the base of each leaf. The springing back of the free ends of the perianth leaves takes place at the moment when the "ringing" of the flower has reached its maximum. The tips of the perianth leaves press against the pedicel and the pressure so generated lifts the flower and throws it with

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some force into space. As the flower moves through space, the anther sacs split open and the pollen grains escape forming tiny clouds of "dust." In such a manner pollination of the female flowers on female plants is brought about.

My observations on the same plant do not support von Wettstein's contentions for the following reasons:

(1). Functional nectaries are present in large numbers on the male inflorescences.

(2) Many insects visit the male plants attracted by odor and pollen.

(3) Male flowers discharge pollen before falling off.

(4) Male flowers remain attached to the plant after the perianth leaves have been recurved.

(5) Female and hermaphrodite flowers also possess the same kind of abscission zone.

(6) Thousands of male flowers examined showed no extreme reflexing of perianth leaves.

The flowers of *Mercurialis annua* in common with a number of other forms show a precocious fore-shadowing of physiological processes. This advance preparation on the part of the plant to discharge a future function certainly indicates that forces besides physical-chemical relations are at work. The purposefulness implied in von Wettstein's analysis of the process that results in the separation of the male flowers from the parent plant loses significance from the fact that female and hermaphrodite flowers also show the abscission zone long before the flowers themselves function. In such flowers the impetus of fertilization cancels and obliterates all the steps for severing the flowers from the mother plant. Unfertilized female and hermaphrodite flowers do not fall off as do the male flowers through a vigorous sundering of parts. They shrivel, dry up and then fall off; the separation takes place at the abscission layer. Whatever the forces in the plant may be that are responsible for the production of a separation layer, they do not draw sex lines. Actual abscission is apparently dependent upon the particular physiological expression of the flower. When the male flower discharges its pollen, it has come to the end of a physiological process which in turn, and as we shall see later, leads to the immediate fulfillment of the rôle of the abscission zone.

The literature on the abscission layer in plants is extensive. For a comprehensive evaluation of that phenomenon I refer to a work of Pfeiffer (1928) entitled "Die pflanzlichen Trennungsgewebe."

When a flower falls off, a surface is exposed from which substances could readily escape. The safeguards to prevent such loss can be studied and analysed long before the flower drops off. Between the time of the

delimitation of the cells concerned in the abscission phenomenon and the complete girdling of the pedicel, a considerable number of cells undergo change in form, function and position. It is apparent that such changes cannot be directly observed as cell motions, but if it were possible to photograph these cells step by step during the time when all the preparations towards the throwing off of the flower go on, one could by projecting the photographs and accelerating the speed of the intervals, secure evidence to show cell movements of a pronounced kind.

In studying the pedicels, cytologically and histologically, in all stages of development I have been able to reconstruct a dynamic picture of the process. The process involves cell differentiation, cell multiplication, the

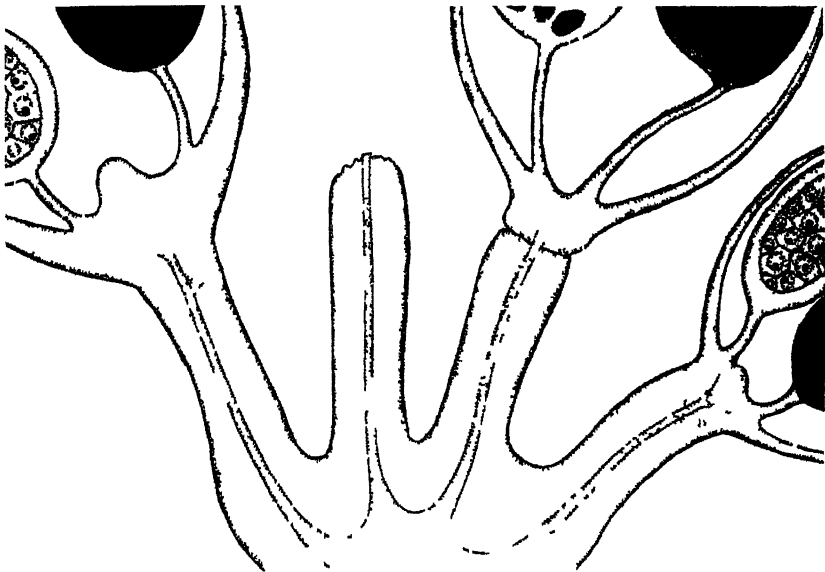


Fig 1

formation of a definite tissue, cell movements, cell adjustments, the progressive destruction of cells, and finally the synthesis of a fluid which passes out of the pedicel after the flower has fallen off and acts as a plug preventing the escape of liquids

Figure 1 shows diagrammatically four pedicels in various stages of development. The stippled areas of the two extreme ones show the distribution of the cells that are concerned in abscission. The inner flower to the right shows an advanced stage of the process, the constriction has reached the vascular strand. The fourth pedicel from which the flower has dropped

off shows a ragged end made up of dead cells covered over by the exudation from the stalk itself

Figure 2 shows a portion of a male flower in the early stages of preparation for abscission. The conspicuously large vacuolated cells are those at the base of the perianth leaf which according to von Wettstein, are concerned with the mechanism of forcibly turning the perianth leaves sharply backward. Immediately below them are the cells of the pedicel

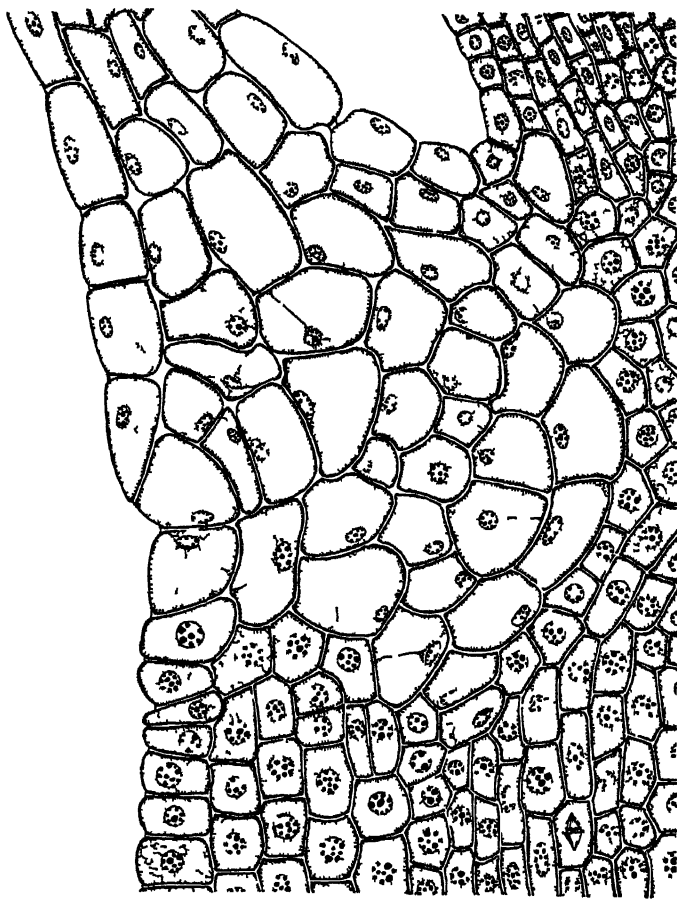


Fig. 2

proper, many of which are meristematic. The two cells of the epidermis that are most compressed show the position of the constriction ring which will move inward like an invisible strangling thread. The other epidermal cells show no form of modification. Next to the epidermal layer division of cells takes place which results in increase in length and circumference of the pedicel as a whole. The meristematic cells have an homogeneous

non-vacuolated cytoplasm in contrast to the non-meristematic cells which are vacuolated. The nuclei of the cells, whether spherical or elongated, exhibit the persistent prochromosomes of *Mercurialis annua*. In a more advanced stage the region above what is to be the abscission layer, grows more rapidly in circumference than that below, giving to the base of the flower a cup-shaped appearance. Large intercellular spaces are present in the perianth leaf tissue and none are present in the pedicel tissue.

Figure 3 shows a portion through a longitudinal cut of a pedicel. It is now wider on top than at the bottom. Cell division goes on actively throughout the whole of that particular region. Fibro-vascular differentiation takes place amidst active nuclear and cell division. The cells to the right and to the left of what is to be the vascular bundle show a striking balance in the rhythm of division. Apparently the impulse that sets the cells of the abscission layer into activity must have travelled radially and must have affected all parts on the circumference at the same time. It is

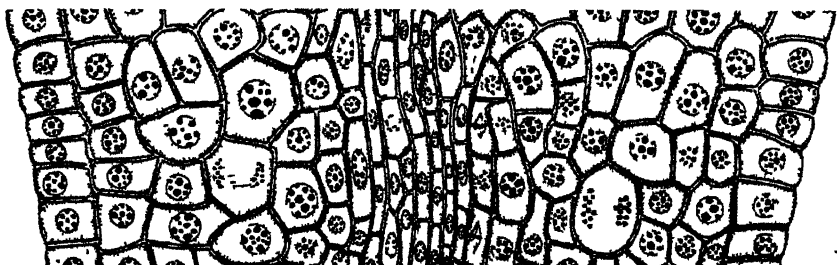


Fig. 3

worth while noting that at this point there is no definite orientation of cells; that occurs later. The two conspicuous cells in the anaphasic stage of nuclear division will of course be divided into two cells whose long axes will be at right angles to what is to be the abscission zone.

Long before the flower opens, preparations for its removal go on. Not only are cells differentiated in the region where the actual constriction will take place but also in the regions below and above. In figure 1 the densely stippled areas of the two outer pedicels show those regions. The value of cytological preparations is apparent when the full length of the pedicel is examined. The contrast between the cells actively concerned with abscission and the rest of the cells, emphasizes how killed and stained material may be an index to profound physiological functions. By a study of these dead cells arrested in their activities a plausible story can be pieced together. The cells concerned in abscission differ from the other cells in size and form and in the appearance of their cytoplasm and nucleus, and

perhaps of even greater significance in the case of *Mercurialis annua*, in the exaggerated size of the prochromosomes that are ever present. Figure 4 shows several of the cells concerned with abscission. The dense and homogeneous cytoplasm is non-vacuolated in contrast to that of the neighboring cells whose cytoplasm is characteristically vacuolated. Such a homogeneous cytoplasm common to all of the cells concerned with abscission speaks for a specialized kind of activity. Equally striking are the prochromosomes and the nucleoli. The appearance of the cytoplasm and

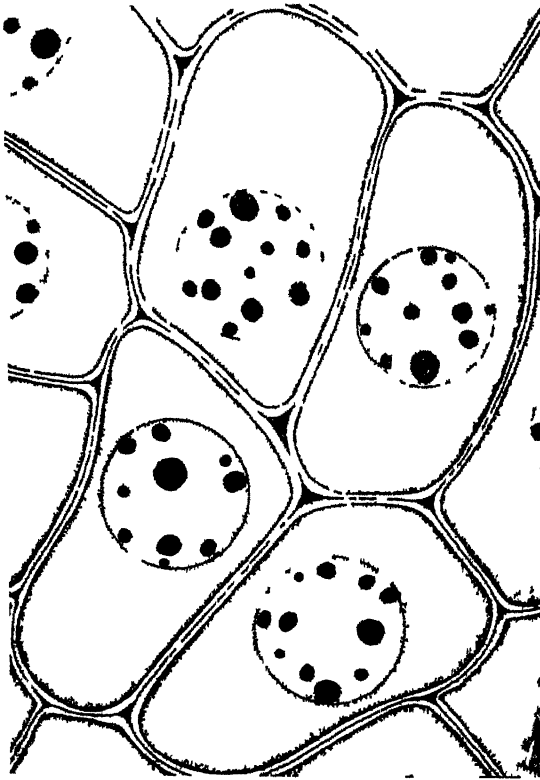


Fig. 4

the nuclei of these cells mark them as especially favorable to the interpretation that the whole cell is actively at work. The prochromosomes are permanent structures. They lie for the most part close to the periphery of the nucleus. They show variability in size. The size of the nuclei and the apparent superiority of the size of the prochromosomes over those of neighboring cells, show that the physiological activities of the cell have a reciprocal influence upon the morphological expression of the parts concerned in carrying out their rôles. Prochromosomes, nucleoli, nuclei and

cytoplasm through their size and texture indicate the tasks that they perform. The four cells indicated in the text figure are representatives of those cells that take part in the abscission phenomena and when a large number are seen at one time, they proclaim their function. The nucleolus is the largest of the bodies in each of the nuclei.

When the meristematic cells of the abscission zone have lost the power of further division, a special orientation of the cells becomes apparent. How that is brought about I cannot say. It must be remembered that simultaneously with the activities that are going on in the pedicel, the parts of the flower which it subtends have gone on in their differentiation. The interdependence of parts must not be lost sight of. It must be re-

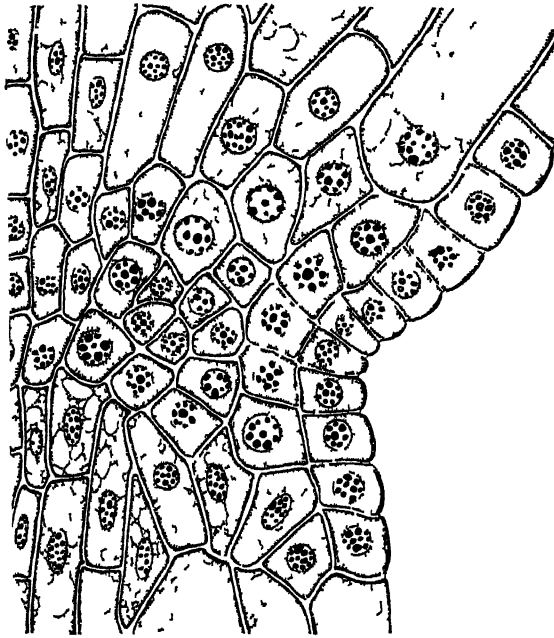


Fig 5

emphasized that the fore-shadowing of the process of abscission is not an accidental thing. The program included the structures above and below the line of cleavage. It is logical to assume that communication between the parts concerned is always maintained whether it be through movements of substances, through change in pressure relations, through growth hormones, or through impulses travelling from cell to cell. Shifting and readjustment of cells takes place in the abscission zone, especially in that region through which the plane of cleavage is to pass.

Figure 5 shows a portion of what is to be the actual region of cleavage

Cell division has stopped. The cells with dense and homogenous cytoplasm are now more restricted. The casualness of the arrangement of the cells seen in figure 3 has given place to an orderly orientation. Epidermal cells are of two kinds: cubical ones with homogeneous cytoplasm and greatly elongated ones poor in cytoplasm. The intercellular spaces have increased in size. A dissolution of the cementing substances which had kept the cells rigidly stationary prior to that event must have occurred. The enzymic fluids by digesting the binding material created more room for the closely packed cells. I am convinced from an examination of a very large number of stages that the cells, like individuals released from very cramped positions, stretch and shift and achieve new adjustments in their positions. Harper (1918) has shown in his studies of *Pediastrum* that in the colonial organization of the swarm cells the intercellular spaces are not always uniform, some are larger, others smaller, yet a balance is achieved. The

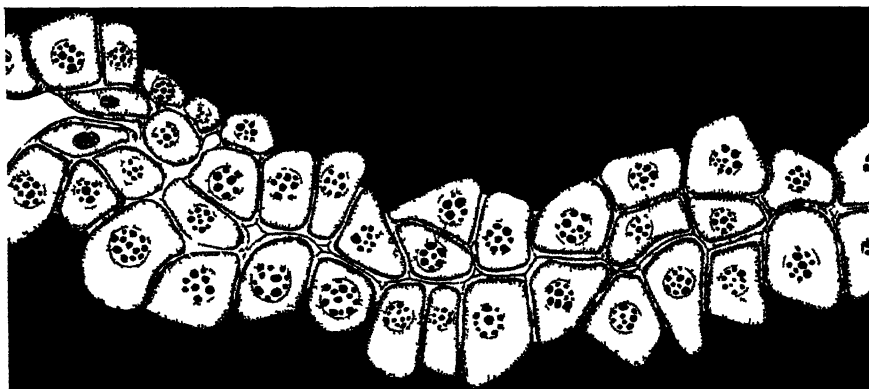


Fig 6

balance is achieved after a series of trials and errors upon the part of the individual cells until a stable plate is established. In the pedicel of *Mercurialis annua* flowers the cells which are in a more circumscribed environment, hemmed in as they are by other cells, arrive at a stable equilibrium in a manner that is described for *Pediastrum*. As in *Pediastrum* the large number of empty intercellular spaces are not uniform, some are large and others are small.

The actual sundering process starts at the epidermal cells of the pedicel and progresses towards the vascular strand in the manner of an invisible strangling cord. Actual separation is accomplished by two distinct methods: the progressive death of the cells and the destruction of their middle lamellae. Figure 6 shows a portion of the cleavage plane. To the left are two cells that have died. There is no difficulty in recognizing such cells

because the nuclei no longer show differentiation of their contents. All the cells of the layer to which the two above mentioned cells belong have died simultaneously. The cells that are the first to die are the ones with shorter vertical diameters which we saw in figure 2. The middle lamella between the two has disappeared and a gap is formed between them. Intercellular spaces become decidedly more pronounced. Several may coalesce to form a single large one. Death of cells and the production of a cleavage furrow do not necessarily coincide since the cells in the vicinity of the large intercellular spaces have all the appearances of living cells. When the cells next to the dead ones die in turn there is a further coalescence of cavities and great inroads are made upon the circumference of the pedicel leaving the floral structure above in a less firmly held position. The cytoplasm of the cells is still homogeneous but perhaps not as dense. The same brilliancy of staining characteristic of all the cells concerned with abscission, persists in the remaining cells indicating an unabatement of special physiological activity. The orientation of cells shown in figure 5 has reached its climax at the stage shown in text figure 6.

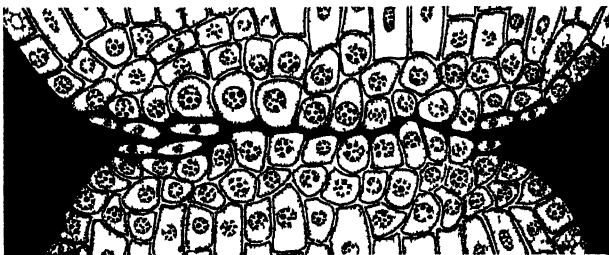


Fig. 7

Figure 7 is a tangential section of the pedicel in a still more advanced stage of cleavage. There are many more dead cells and the split is well on the way towards completion. In this figure the specific orientation of the cells along the cleavage plane is very pronounced. The dissolution of the middle lamellae goes on simultaneously with the dying of the cells. Arrived at the fibro-vascular bundle, the way is barred and at that point the process comes to an end. The final act of cleavage is a mechanical one. By that time the perianth leaves have unfolded and the anthers have dehisced or are about to dehisce. The slender bundle can barely hold the top-heavy burden. The moment comes when the flower snaps off breaking across the conducting cells.

It must not be imagined that the cut is a clean one; we have seen in figure 6 that the cleavage plane, while it tends to remain horizontal, wavers slightly up and down and the result is an irregular surface.

Figure 8 shows a pedicel from which the flower has fallen off. It is composed of dead, dying and living cells. The dead cells are distorted and have cell walls that are greatly swollen. The swelling of the cell walls must have occurred prior to the time the flower fell off since there are no cell contents. To the left and on top, one cell shows a nucleus in the process of disintegration and the cell wall in the process of swelling. The cells below the dead ones are intact and alive. It is interesting to note that the live cells in contact with the dead cells are no longer subjected to reciprocal turgor pressure and their ends have become convexly rounded.

Dead and swelling cells are not sufficiently water-tight to prevent liquids from escaping. Provisions for such an emergency have been made

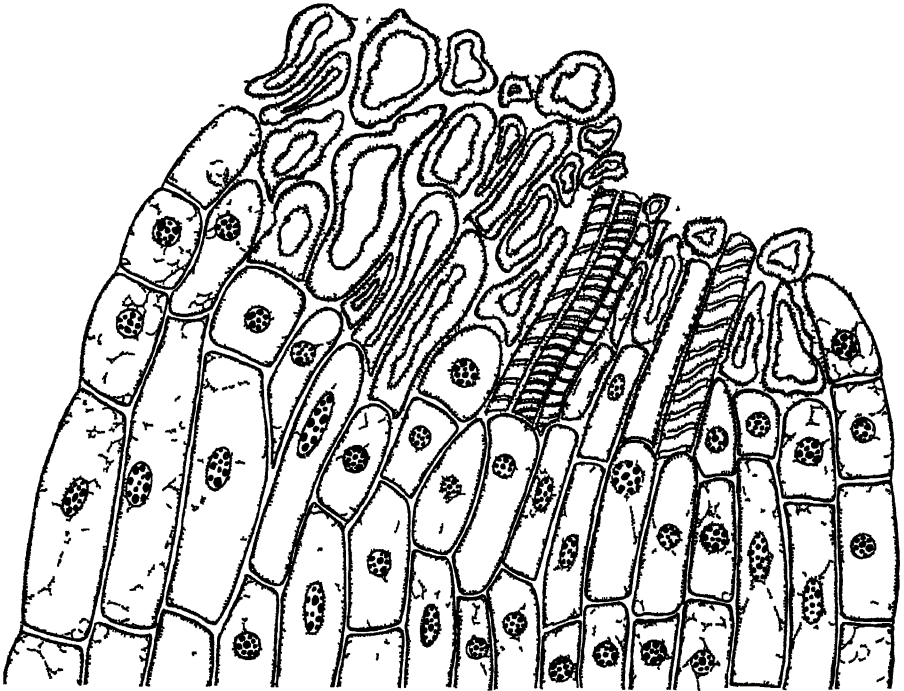


Fig 8

in the form of a sticky coagulating fluid which effectively plugs up the opening immediately after the break occurs. Here we have a situation analogous to the action of the fibrinogen of the blood. That the substance which escapes from the free end is sticky is evident since pollen grains are often caught and held there. The stippled area surrounding the dead cells indicates the extent of the exudate. Upon coagulation the sticky material shrinks.

In the phenomenon of abscission in *Mercurialis annua* we have the evidence that there is a precocious preparation for future physiological functions. The preliminaries as I have stated before, involve female and hermaphrodite flowers as well as male flowers. Once the process has started it continues but comes to a halt in female and hermaphrodite flowers; it proceeds to its logical conclusion in the male flower when that flower has fulfilled a physiological task, namely dehiscence of the anthers. The process of throwing off a flower involves the production of a large number of cells that take part in the various steps in abscission. It involves also a readjustment, a shifting and realignment of cells. It involves an orderly marshalling of two groups of cells lined up facing one another whose destruction forms a cleavage gap. In the plant of *Mercurialis annua* the casting-off of male flowers is a form of autotomy. It is not, as von Wettstein maintained, an adjustment to insure wind pollination. It is a phenomenon in which a part that no longer functions is discarded. The plant then is more than the sum of its parts; it is an integrated whole. The precocious stages leading to abscission lend strength to that contention.

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Diplocarpon Rosae: from spore germination to haustorium formation

ALICE ARONESCU¹

(WITH PLATES 16-20 AND ONE TEXT FIGURE)

INTRODUCTION

Previous work on the black spot disease of roses has been concerned with studies on the general morphology and pathology of the fungus causing the disease, and with experiments aimed at the development of suitable methods of control. The purpose of this paper is to present more exact data regarding the conditions favorable for infection, and especially to follow in detail the early stages in the development of the parasite, such as the germination of the spore, the penetration and invasion of the host by the fungus, and the complete development of its vegetative growth. With such facts taken into consideration, it is likely that better methods for control will be worked out, methods which will aim in their application to prevent primary infection as well as the spread of the disease once it has been established.

HISTORY OF THE DISEASE

Fries (1815) describes for the first time the conidial stage and names it *Erysiphe radiosum*. Libert (1826), in France, reports the fungus on *Rosa turbinata*, naming it *Asteroma Rosae* N.

Fries again (1828) describes the fungus under *Erysiphe radiosa* and characterizes it as having a fibrillose radiating mycelium. In 1849 he changes the name to *Actinonema Rosae* (Lib.) reporting the mycelium often sterile and giving a more complete description of the delicate "perithecium" and the two-celled spores. In Rabenhorst's "Deutschlands Kryptogamen-Flora" (1884) the fungus is found under *Asteroma radiosum*. Bonorden (1853) describes it as a new species, *Dicoccum Rosae*, without mentioning any of the earlier reports. Saccardo (1884) accepts *Actinonema Rosae* (Lib.), and reports it on *Rosa centifolia*, *R. gallica*, and *R. rubiginosa*. He gives its distribution as Sweden, France, Brittany, Italy, Belgium, Germany, Austria, Lusitania, and North America.

Trail (1888) in Scotland, describes the fungus on *Rosa tomentosa* as *Marsonia Rosae*, a new species. Independently of Trail's report, Briosi and Cavara (1889) mention the fungus on *Rosa hybrida* under the name *Marsonia Rosae* (Bon.) Br. et Cav. Saccardo (1892) notes that Trail's

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Marsonia Rosae is the same as *Marsonia Rosae* (Bon.) Br. et Cav. and that it does not differ from *Actinonema Rosae* previously described.

Scribner (1887a) gives a full description of the disease as it appears in the United States. He reports a series of observations regarding the susceptibility of the leaf in relation to its age and roughness, describes the yellowing and early defoliation caused by the fungus, and realizes the fact that because of defoliation the plant is forced to put out new leaves continuously, a condition which brings it to an exhausted state for the following year. He reports his observations on climatic conditions favorable for infection, on the spreading of the mycelium through the leaf, browning of the leaves, overwintering and control.

Humphrey (1889) pointed out that the perfect stage of the fungus is probably saprophytic on the overwintering leaves. Maynard (1889) undertook experiments on controlling the disease. He found the evaporated sulphur method successful in the greenhouse. In 1895 McAlpine noted the occurrence of the disease in Australia. Halsted (1902) reported having observed the disease in 1892 on *Rosa humilis*, a wild species.

The first detailed scientific study of the organism is made by Ducomet (1907). After a brief statement regarding susceptibility and a description of the symptoms and the effects of the disease, he pays more attention to the later stages of the invasion of the host by the fungus. He illustrates the manner of association of subcuticular hyphae to form the system of strands characteristic of the fungus. Good descriptions and illustrations of the formation of acervuli are also given. Ducomet pictures the fungus as sending "intracellular hyphae" into the host cells and reports the absence of haustoria.

Wolf (1912a, 1912b, 1913) discovered the perfect stage of the parasite and proposed the name *Diplocarpon Rosae* Wolf. Through artificial inoculations starting with ascospores he connected both stages of the fungus. The importance of the ascospore stage in carrying the fungus over winter is stressed. He also noted that the ascospores do not germinate unless in contact with the rose leaf.

Chiffot (1914) observed that the petioles, stipules and the stem at the base of the diseased leaves may also show infection at times and that even sepals and petals are attacked, a fact confirmed later by Green (1931). Chiffot is of the opinion that the fungus can penetrate quite deeply into the tissue of the stem and infect the young axillary buds which could spread the disease when grafted.

The seriousness of the disease in greenhouses during winter and its relation to temperature and moisture was brought out by Massey (1917).

He determined also (1918a) that the amount of defoliation varied directly with the percentage of infected leaves.

Alcock (1918) and, more recently, Green (1931) mention the fact that the perfect stage of *Actinonema Rosae* cannot be found in Great Britain. They also give a good account of the varieties that show infection on young stems. According to Alcock the spore-bearing acervuli in the young shoots are of two kinds; some develop immediately beneath the cuticle and others are formed in deep cortical cavities. Both authors state that the spores are not found in two-year old acervuli. Sections of young infected shoots of the very susceptible variety Juliet analyzed in January and February (Green, 1931) revealed acervuli full of spores which germinated in water at 24°C.

Other papers on black spot have appeared from time to time (Massey and Westcott, 1928; Massey and Parsons, 1931; Dodge, 1929, 1932; Howitt, 1930; White, 1930a, 1930b, 1932; and Green, 1931). These authors discuss particularly susceptibility and climatic conditions in connection with infection and they also give results of their experiments on different methods of control.

In a comparative study of the strawberry leaf scorch fungus, *Diplocarpon earliana*, and the black spot fungus *Diplocarpon Rosae*, Wolf (1926) corrects certain statements previously made, and also adds some interesting facts. He states, in this paper, that the hyphae of the parasite are intercellular and that "oval to elliptical haustoria penetrate the host cells." He describes, the first time for this fungus, spermatia that may be found in spermogonia at any time during the period from October until early in January. The spermatia may arise in separate acervuli similar to those which bear conidia, or they may be formed in the same acervulus with the conidia.

Dodge (1931) reports that inoculation experiments show that even Red Radiance may be readily infected in the greenhouse. Spotted leaves which had overwintered in wire cages, were inspected by him two years in succession without his finding any ascocarps. He shows conclusively that the mycelium is intercellular and not intracellular, and that numerous rather long haustoria are present especially in the epidermal cells.

SYMPTOMS OF THE DISEASE AND LIFE CYCLE OF THE FUNGUS

The disease can be recognized from the beginning of its development by dark spots with fringed borders about one centimeter in diameter which appear mostly on the upper surface of the leaves. Pale brown spots, with a few acervuli, can sometimes be seen on the lower surface of infected

leaves. The irregular border of the spot is due to the ramifying strands of parallel hyphae which can be seen in young spots radiating from the points of infection.

Alcock (1918), Massey and Westcott (1928), and Green (1931), give detailed lists of Hybrid Teas, Climbers, Hybrid Perpetuals and soft-wooded varieties with stem infections. The variety Los Angeles is more subject to stem infection than any other rose in the New York Botanical Garden. White (1930a) reports also heavy stem infections on this rose and also on Etoile de Feu.

Massey (1918a) and White (1932) show that defoliation is directly correlated with the amount of infection. The yellowing may be confined to the region immediately surrounding the spot, or it can extend much beyond the tissue actually invaded. Yellowing and defoliation seem to vary with the different varieties, with the intensity of infection in the same variety, and with the number of spots on the leaf. In the case of Golden Ophelia the chlorotic condition appears soon after infection and defoliation is abundant. Red Radiance, on the other hand, does not show much yellowing of the leaves, which may remain on the branches a long time after the disease has manifested itself. Grieve (1930) is of the opinion that because of the defoliation the plant becomes starved, and a "die-back" condition begins.

Acervuli are found on the affected parts a few days after the appearance of the spots. They occur mostly on the upper surface of the leaves and only rarely, and a long time after the infection, are they found on the lower surface, in which case a brown spot is evident. They are covered by the cuticle which ruptures irregularly.

At about the end of October and until about January, spermatia are formed (Wolf, 1926) either together with the conidia, filling up the upper part of the acervulus, or in separate fruiting bodies. Late in the autumn, following defoliation, the mycelium invades the deeper layers of the dead leaf and forms a stroma between the epidermis and the palisade parenchyma. Wolf says that archicarps are formed in great abundance during October, and were noted by him also in November and December. Apparently, if all conditions for fertilization (if such is necessary) and for the development of ascocarps are provided, apothecia with asci may be expected in the spring.

The apothecia open by rupturing. The ascospores are not projected violently from the asci; they merely pile up in a whitish heap in the opened ascocarp. Apparently they are spread by wind and rain. If unfavorable conditions for formation of asci are present (Dodge, 1931), these deep-seated fruiting bodies are filled with apothecial conidia cut off in the early

spring from the upward growing filaments. In the spring of 1933, the writer found but very few ascocarps either on the decaying leaves from the rose garden, or on artificially inoculated leaves that had overwintered in wire cages. Many subepidermal acervuli with apothecial conidia, however, were found on almost every infected leaf. Scribner (1887a) is of the opinion that the new shoots in the spring are infected by the spores lodged upon buds at the bases of the petioles, by the water trickling down the leaf-stalk. Frank (1896) says that the mycelium can develop saprophytically from new acervuli which can overwinter in that condition, and discharge the spores in the spring. Wolf (1912b) could not find, in the winter time, acervuli which were bearing conidia. Green (1931) states that on one year old stems acervuli are formed in large numbers in the autumn but they remain dormant and unbroken during the winter months. We have found in the spring on the same leaves with the subepidermal acervuli, the subcuticular acervuli with spores. Overwintering of the parasite and new infections do not depend, therefore, entirely on ascospores. The apothecial conidia formed in the subepidermal acervuli on the decayed leaves, as well as the conidia from deep-seated and superficial acervuli on infected canes, (Alcock, 1918), can start the new infection in the spring and complete the life cycle of the fungus.

ARTIFICIAL INOCULATIONS

Frank (1896) tried artificial inoculations with the conidia and reported having obtained good spots on the tenth day.

Infections from ascospores were obtained by Wolf (1912b). He observes that germination of these spores takes place in 24 hours, but only on the leaf. The inoculated plants which were kept under bell jars for two days, showed small black areas after ten days. Seventeen days from the time of inoculation, mature acervuli appeared. Inoculations made by the same author in petri dishes lined with moist filter paper, showed the radiating strands within four days. Massey and Westcott (1928) succeeded in obtaining good conidial infections in the greenhouse at about 75°F. The experiments undertaken by Dodge (1931) were mostly for the purpose of seeing whether the nature of its cuticle makes Red Radiance a more resistant variety.

Materials and methods

The writer used potted plants which were stimulated to make vigorous growth by adding fertilizer a few weeks before the experiments were started. The varieties chosen were mostly Radiance and Red Radiance, said to be very resistant, and Felicity, Charles K. Douglas, Mrs. F. R.

Pierson, Henry Ford, and Flammenrose, more susceptible types. Young leaves were usually selected for infection, although older leaves were also inoculated when comparative results were desired. From two to six rings were marked on each leaf blade with India ink. With the aid of a small glass rod, drops of a suspension of spores obtained from acervuli or from artificial cultures, were placed inside of each ring. With a little experience the drops can be made to adhere very quickly to the surface of the leaf so that it is quite improbable that the leaves become injured. In the case of the young leaves of Red Radiance it was somewhat more difficult to make the drops adhere. Suspensions of different densities were used without much difference in the general results. The inoculated plants were placed under the "iceless refrigerator" described by Hunt (1919) which provides a fairly saturated atmosphere for the plants. Thus, in most cases, the drops originally placed on the leaves could still be found even three days afterwards when the plants were usually taken out and placed on the benches. The temperature in the inoculation chamber was always from about eight to twelve degrees lower than it was in the greenhouse.

Humidity and temperature

The first inoculation experiments were started merely for the purpose of finding out whether the fungus is heterothallic. It soon became apparent, however, that temperature and humidity were more responsible for the varying results than were the varieties used. In this experiment a large number of leaves of different ages and varieties were inoculated and observations were made in order to determine the length of time required for the first sign of infection to appear. Countings were made for the number of spots in general, for the number of affected leaves according to their age and also according to the variety. About 2200 leaves were individually inoculated in November 1932, in 30 series. An additional 1000 leaves were inoculated in September 1933, in 11 series. Infection spots were noted in all series between the fourth and the sixth day after inoculation.

No important differences were observed when Red Radiance, Felicity, Radiance, Charles K. Douglas and Mrs. F. R. Pierson were inoculated. In the case of Flammenrose and Henry Ford, infections usually appeared from one to two days later. Furthermore, the spots did not seem to increase in size as quickly and remained more restricted in area.

In general, about 92% of the inoculations were successful. Younger leaves showed practically 100% infection. In a few cases infections were obtained on leaves that had ceased growing before inoculation. The spots however, remained very small and no acervuli, or only very few, were formed.

A second set of experiments was started to determine, as far as our greenhouse conditions permitted, the relation between humidity and temperature and the rapidity of infection. For this reason inoculations were made on a smaller number of leaves at convenient rainy periods. Weather conditions two or three days prior to the inoculation were taken into consideration. Inoculations, for example, made on July 12, August 7, and September 25, gave about the same results. The temperature in the greenhouse during these three experiments was, in general, not higher than 92°F. and not lower than 72°F. Each inoculation was preceded by two days of rain or at least partly rainy and partly cloudy weather. Practically 100 % of infection was recorded and good spots showed in most cases the third day after inoculation or on the fourth day, at the latest. In plate 16 is shown a plant of Red Radiance where all the inoculated places showed good evidence of infection on the third day and the spots had developed beautifully by the second week.

Plants in another group were inoculated on July 12, 1933. No rain was registered for the six preceding days and the temperature varied between 89°F. and 102°F. Infection was not visible until the sixth day. In a set of inoculations started on October 5, when the temperature was about 50°F. in the greenhouse, infection was much retarded, and spots appeared variously from the fifth to the eighth day.

Another series of experiments was conducted to determine whether it is sufficient merely to provide a humid atmosphere or whether the spores must actually be in a film of water. One plant was inoculated on July 10, and was left on the greenhouse bench so that the drops dried out immediately. The atmosphere was quite dry, no rain being recorded during the previous three days. No infection occurred. On August 21, after two rainy days, the same experiment was repeated with four plants. One plant was left out in the greenhouse bench so that the drops dried out about an hour after inoculation; another was left on the bench until after the drops dried out, and then it was placed in the inoculation chamber; a third was left for about 20 hours in the damp chamber and then taken out; the fourth plant was kept in the damp chamber three days as usual. In the case of the first plant where the spores must have germinated in the humid atmosphere of the greenhouse, the spots appeared on the fifth and sixth days. The other three plants showed good infections on the third day. It is apparent from these series of experiments that humid atmospheric conditions, under favorable temperature, are sufficient to provide germination of spores and infection.

Under favorable conditions, large numbers of acervuli appear about nine days after the inoculation is made. The mycelium advances gradually

into the lower tissue of the leaf, so that a brown spot can be seen on this side about two weeks later, but acervuli, if they appear here are developed about a month later and in small numbers.

Good infections, under the same conditions as cited above, were obtained by placing the inoculum on the lower side of the leaf. The percentage of infection is a little lower, due probably to the more unfavorable position of the spores in the drop of water. When infection does follow, a light brown spot is seen on the third or fourth day after inoculation. The mycelium progresses rapidly towards the upper side of the leaf where a distinct but more irregular spot develops and acervuli appear as early as the eleventh day after inoculation, while on the lower side, where the inoculum was actually placed, they did not form until after about a month. These results appear very interesting from the point of view of physiological conditions required by the fungus to develop in the leaf. Perhaps it is the larger quantity of assimilated substances on the upper side of the leaf that makes the fungus spread and develop acervuli first in this region.

Inoculations were also made on the petioles and the stems. A large number of infected petioles were obtained. Under favorable conditions the spots appeared after about the sixth day. Later on, acervuli with large quantities of spores develop. Inoculations on the stem were not so successful. Only very young stems showed infection on about the ninth day and no difference in susceptibility could be found in the varieties that were used.

Artificial inoculations were tried also in the field. On July 14 at 4 P.M., eighty leaves from eleven different varieties were individually inoculated. the places being marked with India ink as usual. It was a warm clear day. The temperature reached above 80°F. and no rain had been recorded for the preceding eight days. The drops of water dried out almost immediately. The next day was cloudy and rain followed on the second day after the inoculation. Ten days after the inoculation, ten leaves distributed among five varieties showed infection. Another series of infections was made on twenty-five leaves of Red Radiance on September 9 at 12 P.M. A heavy rain, which lasted for about two hours, followed very soon after the inoculation. The next few days were fair or partly cloudy. The fourth day spots appeared on sixteen leaves showing that conditions for infection in the field are not much different from those required for infection in the greenhouse. Even after a long rain which followed very soon after the inoculation, enough spores were left on the leaf to produce a good infection. From the preparations of infected material made in order to follow penetration, we observed that even if the drop had not had a chance to dry before the leaves were boiled, many spores still stuck to the surface of the leaf.

Light

Other experiments were made to determine whether plants kept in total darkness could be infected. One inoculation chamber was placed in a photographic dark room, and another one, for controls, was placed in the light, close by the dark room. The temperature registered by minimum and maximum thermometers placed very near the plants showed only very slight differences in the two chambers.

Three series of experiments were made. In the first two series the plants were removed from the dark room three days after inoculation and taken with the controls back to the greenhouse. Infection appeared on the fifth day on the controls and on the sixth day on the plants kept in the dark. The amount of infection was a little higher for the controls. These small differences were not considered important, because they may also occur among different plants inoculated under the same greenhouse conditions. In the third experiment the plants were left seven days in the dark room. No defoliation occurred and no marked chlorotic condition was observed. The plants showed good spots and acervuli developed after the usual interval of time.

Another experiment was made in order to observe whether infection would appear earlier if only the infected leaves were kept in the dark while the rest of the plant was exposed to light. For this purpose the inoculated leaves (including the petioles) were covered with tinfoil. Good spots appeared first on the fourth, and others on the fifth and sixth days, while the controls in the inoculation chamber showed infection, in general, either on the fourth or the fifth day.

Fromme (1913) has shown that total light exclusion either early or late in the incubation period of *Puccinia coronifera* on oats, completely checks the growth of the fungus for the length of time the host was kept in the dark. He explains this retardation as due mostly to the lack of assimilated food and not to the total inability of the fungus to develop for a short time in the dark. In our case it seems that the fungus can develop very well in the dark and that even the reduced supply of food in the leaf is still sufficient for the parasite.

The inoculation experiments, in general, suggest that the best conditions for infection are a saturated atmosphere and a medium temperature of about 75°–80°F. In these cases, spots appear as early as three days after inoculation. Under these conditions, as will be noted later, infection is assured if the spore is provided with a humid environment merely for the first fifteen hours.

INFECTION IN NATURE, RESISTANT VARIETIES, IMMUNITY

All those who have worked on black spot control and have kept records on the degree of infection of the same variety through a few years, agree that there is no completely immune variety. They also agree that immunity and seriousness of infection depend to some extent on climatic conditions.

Numerous and often contradictory records on susceptible varieties are found in the literature. Scribner (1887a) and Frank (1896) report that climbing roses and moss roses and those with rough leaves and hairy and thorny stems seem to suffer more than other kinds. According to Ducomet (1907) *Rosa gallica*, *R. centifolia*, *R. rubiginosa* and *R. indica* exhibit a marked degree of resistance. Chiffot (1914) states that all kinds of roses can be attacked. Green (1931) mentions in passing, that the thin leaved varieties are the most susceptible and thus are the first attacked. The roses with thicker and tougher leaves resist infection better up to the middle of the season when they may become more or less spotted.

Massey (1918a) is the first one to make careful observations on resistant varieties from his extensive experiments on black spot control undertaken at Cornell University and also at different nurseries. In 1928 he considered the Hybrid Perpetuals and the Pernetiana group the most susceptible. Later, however, Massey and Westcott (1928) place the Austrian Briers and the Polyanthas at the head of the list.

Dodge (1932) reaches the conclusion that probably no variety is really immune to black spot. From our own observations of plants in the same rose garden made during the summer of 1933, one should not place too much confidence in reported "resistance." There are certainly different degrees of resistance among the Climbers, however. Star of Persia, a yellow rose, was very badly diseased while other varieties like Paul Noel, Ben Stad, Mary Lovett and Daybreak which grew near it, remained free from infection. After a period of a few successive and quite long rainfalls even Red Radiance showed a number of spotted leaves. One might question whether the little resistance shown here is due to internal conditions in the plant or whether the infection is usually impossible because the waxy substance on the surface of the cuticle normally prevents drops of water from adhering to the young leaves. The writer has always found it more difficult, in making the artificial inoculations in the greenhouse, to make the drops of spore suspension adhere to young leaves of this variety.

Some varieties may escape infection as pointed out by Massey and Westcott (1928), and "although there is doubtless evidence that varieties differ in degree of susceptibility, it is difficult to classify on this criterion,

based only upon observations, because the factor of klandusity varies with the season, locality, and even the location in the garden."

PENETRATION, INVASION AND HAUSTORIA

Review of the literature

In the case of the fungi that penetrate the unwounded tissue of the leaf instead of entering through the stomatal openings, a much more complicated process must be explained. There are many factors which work together and make it possible for the fungus to penetrate through the outer wall of the epidermis.

There seems to be a general agreement that in order that penetration may be possible, an intimate contact between the hyphae and the host is necessary. This is better accomplished, in most cases, with the aid of an appressorium. The first one to observe the function of these organs was Frank, who named them "Haftorgane" (1883), and showed that they were not "secondary spores" as considered by Fisch (1882), Scribner (1887b) Halsted (1892) and others. De Bary (1886) in his study of the disease caused by different species of *Sclerotinia* is of the opinion that two conditions are necessary for the formation of these organs. There must be a mechanical stimulus produced by the contact between the hypha and some hard substance such as glass of the culture chamber, or the cuticle of the host, and that the fungus must grow in a film of water and not in a nutrient medium. If the spores are germinated in nutrient solutions, penetration occurs also, but no appressoria are formed. His explanation for this is that the solution favors the formation of a larger amount of toxic substances which permit penetration immediately after the hypha has touched the epidermis.

Ward (1888) showed that the tips of the hyphae of *Botrytis cinerea* when coming in contact with the cover slip flatten out and form appressoria. Büsgen (1893) in the case of *Fusicladium pyrinum*, Miyoshi (1895) and Hasselbring (1906), are in this respect, of the same opinion as de Bary, stating that the contact between parasite and host has the effect of forming appressoria and that nutrient solutions used for the germination of the spores suppress their appearance. Büsgen (1893) and later Blackman and Welsford (1916) observed that appressoria, besides making an intimate contact between host and fungus, probably serve as centers of support during the act of penetration.

Numerous cases have also been found where appressoria were not formed and their absence could not be explained by either of the interpretations proposed above. Boyle (1921) finds appressoria of *Sclerotinia Sclerotiorum* formed sometimes where there are good nourishing condi-

tions. Büsgen (1893), Pole Evans (1907) and Waterhouse (1921) working with different rusts, Blackman and Welsford (1916) with *Botrytis cinerea*, Weber (1922) and Bensaude and Keitt (1928) with other fungi, report in certain cases little or no formation of appressoria under conditions similar to those reported by others to be favorable. All these cases might indicate that it is sufficient to have contact between hypha and host without the presence of appressoria.

We may now consider what were the opinions of different authors as to the means by which the little infection peg penetrates the cuticle and the other layers of the cell wall.

De Bary (1886) after making artificial inoculations with *Peziza Sclerotium* on different plants, came to the conclusion that penetration is accomplished mostly through chemical means. The substance that makes penetration possible is, according to him, secreted by the fungus. This substance contains a series of organic acids, mostly oxalic, which kill the protoplasts of the cells underneath the drop of spore suspension. It contains also an enzyme which has the power to soften and change the chemical composition of the cell walls after the living contents of the tissues have been killed and, in this manner, open the way for the penetrating hypha. Ward (1888) in the study of a lily disease caused by *Botrytis cinerea* and Büsgen (1893) in a series of observations on Hyphomycetes, Erysiphaceae and Uredineae agree somewhat with de Bary. These authors do not insist, however, on the presence of an organic acid which would first kill the cells. Nordhausen (1899) in studies on mosses and higher plants, is of the opinion that the entrance of the penetrating hypha is possible after the decomposition of the cell wall by a ferment which attacks the cellulose. The fungus nourishes itself from the products of this chemical process and then secretes another kind of substance which kills the contents of cells that had previously offered resistance. He could not find any oxalic acid present.

✓Smith (1902) in his paper on "The Parasitism of *Botrytis cinerea*" is of the same opinion as de Bary, namely, that the killing of the cells takes place first. However, he considers the cellulose enzymes to be subordinate in effect to some toxic substance of a different nature, a soluble substance that diffuses through the cuticle and cell walls and kills the cells ahead of the fungus filaments. He has no doubt that this substance is oxalic acid which almost invariably accompanies the growth of *Botrytis cinerea* and is formed in the metabolism of carbohydrates.

Miyoshi (1895) and Gibson (1904) offer a much more complicated explanation for the entire process. From experiments with *Botrytis cinerea* and *Penicillium glaucum* on cellulose and collodion membranes and also on onion scale epidermis, ✓Miyoshi concludes that the mechanical force

exercised by the fungus as it grows, accompanied by the action of some softening enzymes, could make penetration possible. It is of utmost importance, though, that these be preceded by a chemical stimulus which emanates from the host plant. This stimulus is of great significance because it induces the formation of powerful appressoria and infection threads. Gibson (1904) thinks that the chemotropic substance is not a special one because some rusts show good penetration in plants which are not their hosts. The penetrating hyphae die, of course, in these cases before formation of haustoria. The influence of the exosmosis of nutrient substances from the host tissue into the infection drop was partly confirmed in the more recent studies by Brown (1922) and Dey (1933).

As we can see from the above, no difference was made, in explaining penetration, between the chemical composition of the cuticle and the rest of the outer wall of the epidermal cell.

Von Mohl (1847) and de Bary (1887, p. 75) have already considered the outer wall of the epidermis to be formed of different layers among which the top one is the cuticle made of cutin, the intermediate ones are layers of cellulose which are permeated by cutin, and were called "Cuticularschichte" by the first author. The innermost are layers of pure cellulose. Recent studies in the constitution and chemical composition of the cell wall undertaken by Lee and Priestley (1924), Lee (1925), Anderson (1928, 1934) and others, have shown that cutin is a complex mixture of fatty substances consisting mainly of free fatty acids and soaps. Since no glycerol has been identified it would seem that the fatty deposit has more of the characteristics of a wax, rather than of a fat. It is, therefore, more probable that the cuticle could be penetrated more easily by mechanical means and that the enzymes which produce cellulose decomposition could not have the same effect on cutin. In the light of these researches on the composition of the cell wall a series of very elaborate studies on the physiology of parasitism undertaken at the Imperial College of Science and Technology has led to a better understanding of this whole subject of penetration.

Brown (1915, 1916), for example, has shown that the extract from young hyphae of *Botrytis* has a double action: a, solution of certain constituents of the cell wall, resulting in loss of coherence of the tissue, and b, death of the protoplasts. He has evidence that both the lethal and the macerating actions are due to the same substance, an enzyme. The cuticle presents in all cases an impenetrable obstacle to the diffusion of the extract. Microscopical studies carried on by Blackman and Welsford (1916) on *Botrytis cinerea*, Boyle (1921) on *Sclerotinia Libertiana*, and Waterhouse (1921) with infections of *Berberis vulgaris* by sporidia of *Puccinia graminis* are in ac-

cord with Brown's results. They come to the conclusion that the piercing of the cuticle is due solely to mechanical pressure exerted by the germ tube as a whole or by a special outgrowth from it. It is made easier by the presence of the mucilage which holds the germ tube firmly in position and enables it to exert the appropriate pressure. As soon as the cuticular barrier is passed, enzyme action can occur as is shown by the swelling of the subcuticular layers. —

With this discussion in mind, we may now consider the manner by which *Diplocarpon Rosae* penetrates the rose leaf.

Studies on penetration and invasion of the host

Even though one may make many cytological preparations from embedded material, the difficulty of finding a sufficient number of cases showing actual penetration clearly is so great that one is forced to try other methods for this phase of the study. The most satisfactory method for a study of the early stages of penetration was found to be the examination, from above, of the inoculated portion of the leaf under oil immersion. The following method gave the best results.

Drops of spore suspension were placed on the leaves as described previously and, after different intervals of time, leaves were collected and boiled in 95% alcohol for about fifteen minutes to remove the chlorophyll. They were then left in 70% alcohol to regain their softness. The part of the leaf covered by the drop of suspension was cut out and stained in cotton blue dissolved in lactophenol. The fungus only takes the blue stain, while leaf tissues remain unstained. A few hours in the stain is sufficient for showing the early stages of penetration. For later stages, where parallel hyphae are already present, better results are obtained if the pieces are left in the stain for 48 or even 72 hours, because diffusion of cotton blue takes place only through cracked cuticle and through the cut edges of the leaf. The pieces of leaf were mounted in lactophenol, under a thin cover glass. The diffusion of the stain continues slowly so that in a few days the details may be observed much more clearly. The great advantage of this method over the embedding method is that it takes very little time to prepare a number of slides, and each slide prepared shows many stages of penetration which can be observed in detail and in all phases by bringing different depths of the cell into focus. In studying certain cytological details of the formation of haustoria and also of old stages of penetration described in this series, the author has used sections stained with Flemming's triple stain.

The figures, plates 17–20, present a series of drawings made mostly from cotton blue preparations. Our intention in shading the drawings was to show different depths in the cell with the parts of the fungus invading it.

Darker shading represents greater depth in the cell. The contents of the spores and their superficial germ tubes are purposely omitted so as to make it plain that everything not shaded is on the surface of the cuticle. The drawings were made at different magnifications as the case required but the fungus and the host cells were drawn somewhat in their natural proportions. For convenience, proportions were not respected in the text figure. The width of the vertical wall of the host cells varies considerably and appears wider under the microscope at the very top of the cell than it does if you focus a little lower down into the cells.

It was observed that even after boiling and handling the inoculated leaves, many non-germinated spores would also stick to the leaf surface, indicating again that there must be some mucilaginous substance surrounding the spores when they ooze out of the acervulus.

Under conditions favorable for infection, germination is completed within about nine hours after inoculation. Approximately 80% of the spores present on the leaf germinate. Commonly only one of the cells of the spore germinates, and more frequently it is the larger one. Germination may occur at any place in the cell wall but generally appears either at the tip of the cell (pl. 17, fig. 1, a) or at the middle, near the separating wall (pl. 17, fig. 1, b). The cell that is ready to germinate swells and the spot where germination will take place shows a deeply stained shield around it (pl. 17, fig. 1). Sometimes it looks as if the cell were going to germinate in three places (pl. 17, fig. 1, c).

At these spots, now marked by the stain, the spore sends out a germ tube which varies in shape and size. Sometimes, as shown in figure 2, the germ tube is only a short neck-like outgrowth which swells up immediately into an appressorium. Figure 3 of the same plate shows a series of germ tubes of varied shapes and increasing lengths before the formation of the appressoria. Long germ tubes are very seldom found (fig. 3). Very long superficial germ tubes will fail to make an appressorium and probably will never succeed in penetrating (pl. 17, fig. 5, a). In the case of *Stagonospora Curtisii*, Creager (1933) shows that the very long and several times septate superficial germ tubes form an appressorium, thus providing for good penetration. Figure 5 shows, at b, an unusual case of germination where one cell has two superficial germ tubes and the other cell, one. Figure 4 shows cases of spores germinated at both ends. The short germ tube forms a penetrating peg, the other will remain superficial. The superficial germ tubes, after having reached a certain length, may lay down cross walls.

Hasselbring (1906) sowed spores of *Gloeosporium fructigenum* in "convex" drops of water on slides kept in a moist chamber. The spores germinated differently according to their position in the drop. Those at the bot-

tom of the drop formed a short germ tube, which enlarged into an appressorium in contact with the glass. The other spores in the drops had germ tubes that floated. The same experiment was made by the writer with spores of *Diplocarpon Rosae*. No differences in germination appeared among the spores of the same drop, and no appressoria were formed.

It may be found that while about 50% of the germinated spores from drops placed on the upper side of a leaf are in a stage ready for penetration, a lower percentage will be found in the case of drops on the lower surface. It is easy to understand why some of the spores in a hanging drop, since they must develop long germ tubes in order to reach the cuticle, are in an unfavorable position for penetration. The method of penetration on the lower and the upper sides of the leaf was found to be the same.

Appressoria may vary greatly in shape as shown in figures 2 and 3. When the spore germinates from the side that touches the cuticle, the appressorium appears circular (pl. 17, fig. 1, d; pl. 18, figs. 10 and 15). These organs are not as conspicuous by their size or shape as they are by the deep staining they take where they come in contact with the cuticle, and by the rays of the dark blue stain which usually surround them (figs. 2 and 3). It is possible that these rays represent cracks made in the cuticle, which is waxy and brittle, by the firm pressure of the appressorium. The stain accumulates in these cracks and forms the blue rays visible around most of these organs. In other cases, instead of pronounced rays, a light blue area is seen immediately surrounding the appressorium (fig. 3).

Diplocarpon Rosae is like certain other fungi in that appressoria are not always formed. In a series of inoculations made in November, all of the preparations showed good stages of penetration from germ tubes which had not formed appressoria. The end of the germ tube showed a light circular spot surrounded by a darkly stained border (fig. 4). These spots which are found also in the center of appressoria (figs. 6-24) represent the so-called "place of germination" of the appressorium where the little infection peg will be sent out to penetrate the cuticle.

In stages older than nine hours, the fungus is seen to have already penetrated the cuticle. The narrow infection peg sent from the germ tube tip or from the appressorium, enlarges into a subcuticular hypha immediately after it penetrates the cuticle. There is no indication at this time of any toxic or enzymatic action of the fungus so we must assume that the cuticle is penetrated by mechanical means. Penetration never takes place through stomatal openings, although guard cells are sometimes penetrated. The subcuticular hypha is very easily distinguished by its vacuolate contents. Figure 6, in plate 17, shows an infection hypha as it is found about

thirteen hours after inoculation.² Text figure 1, A and B, shows the ways in which the penetrating peg enlarges into the infection hypha.

The tapered end of the infection hypha continues to grow a little longer until it reaches the wall between two adjacent cells where it pushes down into the middle lamella (pl. 17, fig. 7). At this stage each infection hypha has laid down a cross wall, a short distance from the penetrating peg (pl. 17, figs. 7-9; pl. 18, figs. 10-17 and 19; pl. 19, figs. 20-24). This wall is very conspicuous because, in most cases when the cuticle is not injured, the subcuticular hypha is stained much darker in that portion near the appressorium. The stain passes down through the infection peg but it can not pass through this impervious cross wall. The importance of this provision will be noted later on.

A careful examination of these infection hyphae shows that while their beginnings can be seen almost in the same focus as the spore, the end portions appear less clear at that focus and in order to follow them distinctly one has to focus lower and lower into the cell. This indicates that while the first portion of the hypha might be strictly subcuticular, the rest of it travels, sooner or later, through the path of least resistance across the cuticularized layers until it reaches the inner layer of the outer wall of the epidermal cell (text figure 1, D and E). Sometimes, the infection hypha, penetrating the two adjacent vertical walls, produces a swelling at the point of entry (pl. 17, fig. 9).

Figure 8 shows a very common case where the hypha does not grow completely across the cell to reach the adjacent vertical walls, but tapering out into a narrow end penetrates directly down through the outer wall of the cell. Some of the infection hyphae may assume peculiar shapes (pl. 17, fig. 6; pl. 18, fig. 10, a). At b, in figure 10, is shown a hypha which apparently has branched immediately after penetration. Another peculiar case is given in figure 20, plate 19, where a very long infection hypha has passed across two adjacent walls. In many cases, instead of being straight, (pl. 17, figs. 7, 9; pl. 18, figs. 12-14, 16-17 and 19), the infection hypha has a wavy outline in passing through the upper wall (pl. 17, fig. 8; pl. 18, figs. 11 and 15). A few cases were found, where, because of the position of the spore, penetration took place through the cuticle of the wall between two adjacent cells so that the infection hypha could be detected in this wall only by two shining blue spots (pl. 18, fig. 18). The penetration of the

² It is necessary to point out here that the hour at which the same stages can be found, varies with conditions of infection. As might be expected many different stages can be seen on the same slide.

infection hypha through the subcuticular layers is believed to take place with the aid of an enzymatic action of the fungus, possibly accompanied by a mechanical process. This point will be discussed in another place.

Fifteen hours after inoculation under favorable conditions, the fungus is able to form a haustorium. The spore (fig. 11) has germinated and has made an appressorium immediately; the peg has penetrated the cuticle and has enlarged into an infection hypha. The hypha has laid down a cross wall (Although at the beginning it is subcuticular, it reaches deeper layers of the upper wall towards the end where it turns down, pushes in between two adjacent vertical walls and, gradually tapering into a fine thread, penetrates the wall ending directly in a haustorium (text fig. 1, D). The young haustorium shown in figure 11, is surrounded by a jelly-like substance which has the aspect of a secretion product. The haustorium appears in the preparations as a fine blue thread all along the distance where it is surrounded by this substance. Its tip end becomes enlarged only after having emerged from this deposit. The swollen wall of the host shown in the same figure remains distinct from the substance deposited around the haustorium. The deposited substance does not take any stain; it is only the haustorium that stains blue, just like the rest of the fungus. Another fifteen-hour stage (fig. 12) shows the gelatinous substance around the haustorium having a more definite form, being now made up of two parts. The haustorium is enlarged into a knob at the end. In this particular case the spore had been washed off in preparing the mount, and only the appressorium with the "germinating spot" is seen at a. A more advanced stage of the development of a "fifteen-hour" haustorium is shown in figure 13, where the knobbed end is much more developed. In this case the wall around the haustorium, and also some distance away from it in the other cell, seemed to be very much swollen and softened and showed a layered structure around the place of penetration. These cases of such exaggerated swellings are very rare. On the same fifteen-hour preparation one can find stages showing completely formed haustoria (fig. 14). The precipitated substance has taken the form of a collar or cup made up of two parts. The haustorium, as soon as it escaped from it, spread out and elongated. About the same situation is shown in figure 17, with the difference that the collar is not divided into two regions.

The fungus can also form a haustorium in fifteen hours by sending down an infection hypha which will penetrate into the lumen of the epidermal cell directly from the outer wall (pl. 18, fig. 15). If the haustorium is vertically oriented that part which comes first into vision, focusing from the top, is the basal part of the collar which appears circular. Focusing lower down, the rest of the haustorium comes into view. These cases

are the best for studying the thinning out of the tapered end of the infection hypha into the thread which penetrates the wall and forms the haustorium (text fig. 1, D and E). In these cases also the haustorium enlarges into a knob-like end as soon as it escapes from the collar-shaped deposit. Figure 16 shows on the lower side of the leaf a haustorium sent in from the outer wall. The haustorium here is more fully developed.

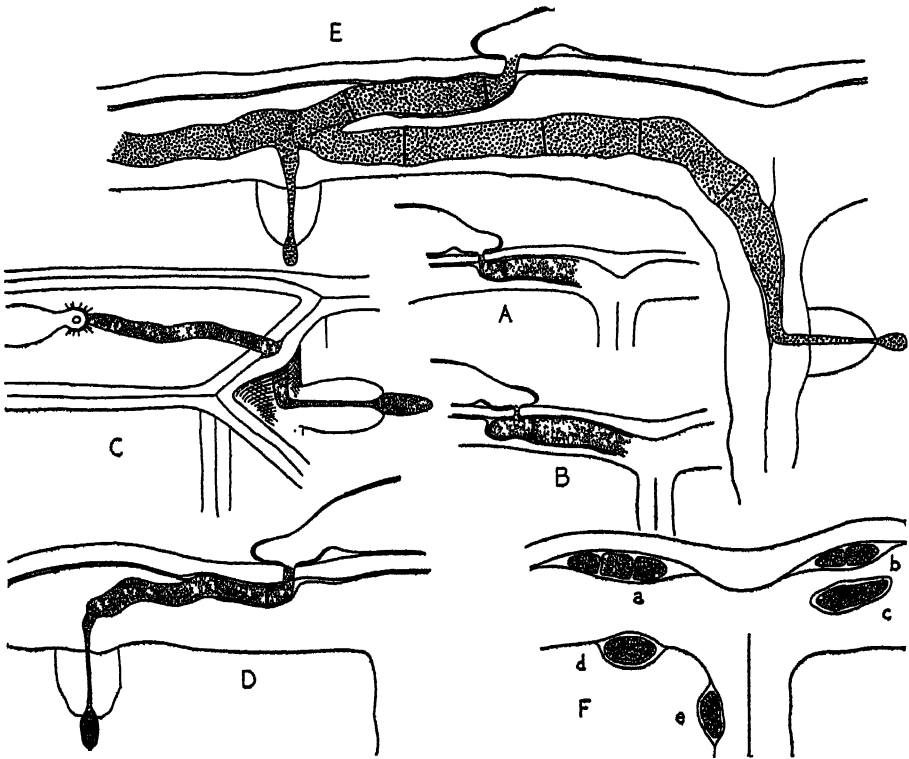


Fig. 1. A-F. Diagrams of various early stages in penetration and invasion of rose leaf by *Diplocarpon Rosae*. Exact proportions disregarded for the sake of clearness. For further explanation see text.

A more advanced stage of invasion is shown in figure 19, plate 18. This is about 29 hours old. The infection hypha has penetrated through the middle lamella and has formed a haustorium at a. Focusing lower down in the other cell a second haustorium comes into view, formed from the same center of infection by the intercellular hyphae which had grown and spread during this time. On the other side of the penetration point the progress of the intercellular hyphae could be observed by two blue spots in the wall. The connection between them was sometimes difficult to make

out because of their undulatory growth following the wall in which they developed. It is interesting to notice that the second haustorium comes from a new portion of the initial intercellular hypha. Usually not more than two or three haustoria are formed by the first penetrating hypha, and these are generally in different cells.

As a further step in invasion, additional hyphae begin to develop on the second day after inoculation. The part of the infection hypha which lies in the subcuticular layers starts to form new branches directly above the place where it has formed a haustorium (text figure 1, E). These branches travel in the upper wall and serve to spread the infection by providing new sources for the development of haustoria. Figure 20 shows the long infection hypha which has passed over the first cell and has penetrated the outer wall of the second cell into which it has sent a haustorium.³ The infection hypha had swollen just above the haustorium and sent out branches on both sides. The original shape of the infecting hypha before swelling is indicated by dotted lines. Similar cases are shown, in figures 21 and 22, except that being a little more advanced, more branching has occurred and the new hyphae are already septate. These hyphae have quite a different aspect from that of the initial infection hypha. They are wider, their cells are shorter and their borders are irregular. Having rich contents and large vacuoles they are easily seen. In figure 21, one of these new hyphae has already reached two adjacent walls and has grown through the middle lamella. The same type of branching can occur where infection hyphae penetrate two adjacent walls (fig. 22). In a more advanced stage (fig. 23), the branching has become quite complicated. One new haustorium is formed at a, and at b and c two other hyphae are ready to form other haustoria. The swelling of the infection hypha takes place here on one side so that its initial form is preserved. An advanced phase of this stage is shown in figure 24. The haustorium at a is the one formed by the initial infection hypha; haustoria b and c were formed from branches developed from this hypha at a point just above the haustorium a. A very unusual situation is shown in the same figure at d. Here the penetrating hypha sent out a branch on the other side of the spore some time after penetration.

None of the stages described above has heretofore been given any attention in studies on the development of *Diplocarpon Rosae*. Not until about 48 hours after inoculation do the parallel strands, which others believe to be the only kind of hyphae representing the vegetative growth of

³ For convenience, the haustoria are represented in some of the drawings only by a circle which is the base of the collar. The little circle in the middle represents an optical view of the haustorial thread.

the fungus, start to develop. Some of the branches of the secondary hyphae that we have just described for stages between 29 and 48 hours do not form haustoria, but produce hand-shaped ends (pl. 19, fig. 21, a, b; fig. 23, d; fig. 26, a). In the case shown in figure 25, the first stages of penetration could not be clearly seen. As the stages become more complicated it is difficult to find the starting point of the infection. It is quite certain, however, that the haustorium at *a* is due to the initial infection. The branched hyphae above the haustorium do not send out new haustoria but both are about to give rise to parallel hyphae. At *b* (fig. 25), the little side branch could grow and produce another hand-shaped formation. Sometimes, as seen at *c*, the finger-like projections have already laid down cross walls.

Figure 26 shows how the first cells of parallel hyphae are formed. At first they are narrow and lay down walls at short and somewhat unequal distances. In preparations stained with the triple stain, the cells are seen to be uninucleate. At the points where branching takes place, they are richer in content (pl. 20, fig. 28, a). A portion where active branching has occurred, starting from a strand of three hyphae, is shown in figure 27, plate 20. Each hypha after a certain distance of parallel growth may branch in turn and add a new hypha to the synema. If the branching continues, one can very often observe strands composed of as many as seven or more hyphae (pl. 20, fig. 32). In this case they are more irregular in shape, and sometimes leave larger spaces than usual between them. A case where two hyphae from a strand have joined another one which had already four parallel hyphae is shown in figure 28. Ducomet (1907) has given detailed illustrations of the formation of these strands as well as an adequate description and it is not necessary to discuss this point further here.

A short distance from the place where the first "parallel hypha system" originates, haustoria of a third series are sent down through the upper wall into the epidermal cells below. Their formation is the same as that described for the very first haustoria which are found at the fifteen-hour stages (pl. 18, figs. 15 and 16). Those formed from the parallel hyphae differ somewhat in shape and size (pl. 20, figs. 28, 29 and 31). In figure 28 is shown a single large haustorium. The vertically oriented haustoria are indicated only by circles. The subcuticular parallel hyphae usually do not send haustoria into cells already occupied by these organs, but, instead, into other free cells. Figure 29 shows at *a*, *b*, *c* and *d* haustoria formed from earlier stages of infection. All of the other haustoria originate from the parallel hyphae, some of them being indicated merely by circles. Two haustoria were never found coming from the same hyphal cell in a synema. Stages like those represented in figures 26 to 29, are about three to five days old.

About the sixth or seventh day after inoculation, new complications arise. The portion of the parallel hypha directly above the place where a haustorium had been formed starts to branch in the same manner as described for the earlier stages represented in figures 20 to 24, plate 19. This branch grows in the subcuticular layers of the outer wall pushing out laterally from the parent parallel hypha (fig. 30, a, b, c). Such hyphae serve as an additional means for spreading infection and for the formation of haustoria. These "scouts" are much wider than any of the hyphal branches of this fungus described before. They have varied shapes, irregular borders and a foamy, cytoplasmic content which, when stained, makes the many large vacuoles more evident. They are clearly the same hyphae that Dodge (1931) refers to as "haustorium mother-cells." They may be either one- or two-celled. These hyphae reach two adjacent walls (fig. 30, c) and immediately form a haustorium, usually in a cell which is free from infection (figs. 31 and 32). Figure 31 shows at a one of these scout hyphae that is ready to send its tapered end directly through the upper wall to form a haustorium. During this stage of infection five or even more haustoria can be found in a single cell (fig. 31).

In order to give a better understanding of the position of the different kinds of hyphae that develop in the epidermal walls a schematic drawing is given at F, in text figure 1. The parallel hyphae at a and b are usually just beneath the cuticle. This is seen in cross sections of imbedded material and can be observed from the top of the leaf, where they appear almost in the same focus as the spores and above all the other hyphae. When they first start to form as shown in figure 26, plate 19, careful focusing indicates that they occupy a lower level in the wall but, soon after, come up directly beneath the cuticle. In cases like those shown in figure 28, plate 20, where one synema passes underneath another, the hyphae composing the lower one are either making their way by pressing down in their growth through the cuticularized layers, or they might be growing right in these layers. The hyphae come up to a higher level again as soon as the "crossing place" has been passed. All the other hyphae borne on the different systems of branching found in the early stages, including the "scouts," occupy a lower position in the layers of the wall as seen in the text figure at F, c, d and e. These observations made from sectioned material are confirmed by careful focusing from the cuticle down into the wall.

MORPHOLOGY OF HAUSTORIA AND RELATION BETWEEN HOST AND PARASITE

An attempt to review all the literature on haustoria seems unnecessary here, as Rice's (1927) detailed discussion and bibliography cover this subject very thoroughly. The writer is concerned here with noting only some

of the earlier papers regarding haustoria, which have been consulted in order to better understand the morphology of these organs as found in *Diplocarpon Rosae*. It seems necessary to discuss here in particular the formation and the meaning of the haustorium "sheath." The term sheath, although attributed by all the workers mostly to the result of a reaction of the host cell against the invading hypha, is not used in the same sense by different authors. Its appearance, time and place of formation differ widely in the various accounts.

When the wall of the haustorium remains thin during its development, some authors report the absence of a sheath. Sappin-Trouffy (1892, 1896) reports thin-walled haustoria in the cases of different species of *Uromyces* and *Puccinia*. Pole Evans (1907) reports no sheath for the young haustoria of the cereal rusts and Dastur (1913) for *Phytophthora parasitica*, also remarks on its absence.

De Bary (1870) in the study of *Erysiphe* says that the sheath-like layer covering the haustorium belongs to the host cell. He considers that this stage represents a haustorium which was checked in its growth. Sappin-Trouffy (1896) reports that *Uromyces Betae* forms haustoria with a much thicker membrane than that of the intercellular hyphae. Mangin (1895) studying the Peronosporaceae states that in all of the species observed the haustoria are never free in the host cells. They are protected by a sheath which does not adhere to these organs. The sheath, according to him, is made up entirely of callose or has some cellulose at its base near the cell wall. It is formed all around the haustorium at the same time that it elongates and thickens in the cell cavity. This formation makes the haustorium appear much larger and with rough irregular borders. Istvánffi and Palinkás (1913) make the same observations in the case of *Plasmopara viticola*.

Ducomet (1907) considers the sheath of *Cuticularia Ilicis* on *Ilex* as a region of digestion, a clear zone with a dark fringed border. Reed and Crabill (1915) report for haustoria of *Gymnosporangium Juniperi-Virginianae* on cedar, an envelope or capsule which stains differently than the fungus.

Smith (1900), in the case of several Erysiphaceae studied, is more explicit in his description of the origin of the sheath. As soon as the haustorium starts to penetrate the cell, an ingrowth from the cell wall begins to form in the shape of a collar. He considers this as an addition of new material to the wall which takes place at the same time with the advancement of the haustorium in the cell. He suggests that the production of cellulose around the region of penetration might be due to some chemical substance excreted by the fungus which excites the cell protoplast to an unusual activ-

ity. As soon as the haustorium has escaped from the collar-like ingrowth, according to Smith, a part of this deposited substance begins to disintegrate. Its products of disintegration, by accumulating between the haustorium wall and the host plasma membrane, make the sheath appear much more definite at this stage. Higgins (1914) describes for *Cylindrosporium* on stone fruits, sheathed haustoria which he thinks resemble those described by Smith.

In many cases sheathed haustoria were reported only for older stages of development of these organs. Tischler (1912), for haustoria of *Uromyces Pisi* on *Euphorbia cyparissias* and of *Albugo candida* on *Capsella bursa pastoris*, Colley (1918) in the case of *Cronartium ribicola* on *Pinus Strobus*, and Moss (1926) for certain species in the *Pucciniastreae*, report sheath formations around mature, old and even dead haustoria. Rice (1927) reports two kinds of thickenings for haustoria of different rusts. She finds some cases where a ring-like thickening is formed some place about the stalk and which she attributes either to a differentiation of the outer surface of the haustorial wall or to a deposit from the host cytoplasm. Some stages in the corn rust show sheath-like thickenings either around the stalk itself or also around the lobes of the haustorium. The formation of this sheath seems to take place almost always around the old haustoria. Rice tries to explain the formation of the sheath as a result of less vigor on the part of the fungus. The sheath is formed very often when the haustorium is forced to enter thick-walled cells.

In the case of some parasitic fungi sheaths in the form of cellulose layers deposited along the hyphae which cross through the cells of their host are also reported. Wolff (1873) in the case of *Urocystis occulta*, Butler (1907) in the case of *Sclerospora graminicola* on cereals, and of *Ustilago Zeae* (1918) on corn, report that hyphae which pass through the cells are clothed with a distinct cellulose sheath, which they consider as deposited by the host cells in a defensive attempt to check the fungus in its growth. Leitgeb (1881) figures them also for the penetrating filaments of *Completozia complens* in fern prothallia. His figures show that he was dealing with true haustoria.

We shall consider now the young stages in the development of the haustoria of *Diplocarpon Rosae* and attempt to identify some of their structures with those described above for other fungi. The first sign of formation of a haustorium is in many cases a swelling of the wall of one of the two adjacent cells, at the place of a penetrating hypha (pl. 19, fig. 25, d, e). The hypha pushes in between the two walls by way of the middle lamella, as can be seen in some of the preparations which show this layer very distinctly. Haustorium mother cells which are quite frequent in the

different rusts, as reported by Pole Evans (1907), Reed and Crabill (1915), Allen (1923a, 1923b) and Dodge (1918, 1922, 1934), are not present in this fungus. The ends of the hyphae thin out into thread-like formations which penetrate the wall, without the formation of a preliminary end cell. At this stage, the fungus sends out a very thin filament which pierces the wall and develops into a haustorium inside the cell.

Very early stages are the only ones which indicate that the plasma membrane is merely invaginated rather than pierced by the invading fungus (pl. 19, fig. 21, c, d; pl. 20, fig. 30, d, e). They also show the haustorium as a very fine thread which has already enlarged at the tip. At first the thread-like part is surrounded by some deposited substance as noted previously, and which is slightly granular and irregularly shaped (fig. 21, d and e). A more advanced stage shows this substance taking the shape of a collar (figs. 22, a, b; fig. 26, b, c). In some cases very little of this substance is deposited (fig. 21, e) so that the haustorium swells up immediately; more frequently the deposit is present in larger quantities and prevents the haustorium from enlarging; only at the tip does the fungus thread escape and swells up as seen in figures 22 and 26. This substance assumes a more definite shape in older stages and can be made out as composed of two parts (pl. 20, fig. 28, b, and fig. 31, b) or as only one part (fig. 28, c). We consider that these formations found around the haustoria of *Diplocarpon Rosae* resemble very much the cellulose collar deposited by the wall of the cell around the haustoria described by Smith (1900). The collar does not increase in size much after it is once formed. It does not seem to disintegrate as reported by Smith for the Erysiphaceae. Sheaths which entirely surround the haustoria have not been observed by the writer.

After the collar has developed completely, the haustorium enlarges and elongates. No lobed or branched haustoria are found in the case of *Diplocarpon Rosae*. They are usually uninucleate and have finely granular contents which stain darkly with cotton blue. A great number of haustoria are sent into the epidermis. They can be found also in the palisade cells in later stages (Dodge, 1931) but were not observed in the cells of the spongy parenchyma.

The haustorial complex formed in earlier stages attains rather large sizes and varied shapes. Including the collar it may be either very long so that it nearly reaches the opposite wall of the cell (pl. 18, fig. 19), or the collar may develop more in width, the haustorium itself thus remaining short (pl. 19, fig. 23, a). In any case, the collar accompanies the haustorium to about half of its length so that not very much direct contact between the fungus and the cytoplasm of the host cell is possible. Haustoria which are

formed in older stages of development, especially those sent out by the "scouts," are very often somewhat smaller. The collar also is less prominent in these cases (pl. 20, figs. 31, 32).

Numerous cases were found where the haustorium was in contact with the nucleus but just as many were noted in which the nucleus occupied a position near the cell wall with no connection at all with the haustorium. Even when in contact with the haustorium, no important deformation was observed as described for other fungi.⁴

Interesting studies made by Dufrénoy (1928, 1929, 1930, 1933) aim to bring out the different changes that take place in the cytoplasm of the host under the influence of parasites, fungi, bacteria and viruses. The most important changes observed in vital stained tissues is concerned with the vacuolate apparatus. He cites different examples of influence of the parasite on the host, such as *Colletotrichum Gloeosporiodes* on *Citrus*, *Peronospora Schleidenii* on onion, *Phytophthora infestans* on potato, *Endothia parasitica* on chestnut, *Uromyces Caladii* on *Arisaema triphyllum*, and *Helminthosporium* on *Hordeum*, and about the same general phenomena are reported. In the case of a cell parasitized either by a hypha or by a haustorium, the single large vacuole, which was present in the cell before the invasion, is shown to break up into small ones. The cell finally appears plasmolyzed. The fragmentation of the vacuome seems to correspond to an exaggeration of the proteolytic processes which is expressed morphologically by an increase in the surface of contact. This fragmentation is nothing but an indication of a reversible evolution from a cell, which was almost at rest, to a period of active metabolism. In the case of some fungi, this fragmentation appears also in the neighboring, non-parasitized cells.

Rice (1934) points out that her detailed studies made on relations of rusts to their hosts have not revealed any changes in the host cell protoplasm around the haustorium. With special reference to *Uromyces Caladii*, she states that no intracellular hyphae were observed for this fungus as represented by Dufrénoy. She also states that *Uromyces Caladii*, like other rust fungi, being a highly adapted parasite makes but little appreciable alteration in the physical condition of the cytoplasm and that no plasmolysis of the host cytoplasm can be observed.

Our observations on this subject in the case of *Diplocarpon Rosae* reveal in a few cases a system of small vacuoles which are gathered around the haustorium in different stages of its development (pl. 20, fig. 30, d, e, f). A much more careful study is needed using vital stains which might bring out more evidence on their presence and origin.

A word regarding the browning of the cells attacked by *Diplocarpon*

⁴ See Rice (1934) for literature on this subject.

Rosae may not be out of place here. Our detailed studies on penetration could not establish a definite time when the browning of the cells occurs, as many variations were found in this condition. The brown spot on the infected part of the leaf can be seen in the more favorable cases as early as three days after inoculation. By boiling the leaf in alcohol, it could, however, be seen in preparations of earlier stages. The microscopical observations sometimes indicate an incipient browning as early as twelve hours after inoculation before the haustorium is formed. Many cases show that the browning of the cells may start immediately after the formation of the first haustorium. Sometimes it is delayed even after the formation of the parallel hyphae. Cells that are invaded by a larger number of haustoria become brown very quickly. In later stages of penetration the infected cells are dark brown and their old and deformed haustoria can scarcely be seen. Wolf (1912b, 1913) attributes the color of the infected spot merely to the browning of the substances in the host cells. We are more inclined to agree with Ducomet (1907) that the parallel hyphae which are also seen to be colored in older stages, probably contribute to the coloration of the spot.

CONTROL MEASURES AS CORRELATED WITH DATA ON INFECTION

Of the "wet sprays" Green (1931) and Parsons and Massey (1933) state that Bordeaux Mixture is the best, although White (1930b) reports that copper sprays seem to produce a dwarfing of the plant in addition to the dwarfing due to the black spot that persisted.

Massey and Westcott (1928), Dodge (1932), and Massey and Parsons (1931) have emphasized the importance of very careful spraying or dusting just before rains. During rainy periods it may be necessary to dust or spray two or three times a week.

Our inoculation experiments have shown that rainy periods with not very high temperature are most favorable for infection. We have shown also that in about nine hours of favorable conditions the spore germinates and that in about thirteen hours after inoculation the fungus has penetrated the cuticle and formed the infection hypha. From this time on, it seems to be able to protect itself against any unfavorable climatic conditions because of an impervious transverse wall which is laid down by the penetrating hypha, just beyond the penetration peg, in the subcuticular layers. By applying fungicides at short intervals of time before precipitation and during rainy periods, the spores can be kept from germinating or from developing infection. We can now better understand why Dodge (1929, 1932) and Parsons and Massey (1933) who have been carrying on experiments on black spot control for several years, agree that the fine

sulphur dusts with arsenate of lead added, are very satisfactory especially if green-sulphur dust, which does not discolor the foliage, is used. White (1932) finds that sulphur sprays adhere well and thus give good control.

DISCUSSION AND CONCLUSIONS

Our experiments have shown that the time required for the spots to appear can be reduced to three days; that infection is very little affected by the absence of light, because the fungus is capable of living saprophytically. The abnormal condition of the plant placed in the dark does not suppress or retard to any great extent the development of the fungus. While Fromme (1913) has shown in the case of *Puccinia coronifera* that total light exclusion checks the growth of the fungus, our results agree more with those of Lauritzen (1919), who concluded that light is not a limiting factor if experiments are not conducted at the border of favorable temperatures and humidity.

The appressoria of *Diplocarpon Rosae* are represented only by the swollen part of the enlarged germ tube, which stains darker at the point where it touches the cuticle. They are not definite organs as described by De Bary (1886) for *Sclerotinia* where they can appear in tufts, or as brown thick-walled spore-like bodies as described by Hasselbring (1906) for *Gloeosporium fructigenum*. Appressorium formation is recorded by Allen (1923a), as taking place on the day following the inoculation. Hasselbring (1906) reports their formation from twelve to eighteen hours after inoculation, while in *Diplocarpon Rosae* they appear as early as the ninth hour.

Nordhausen (1899) and Büsgen (1893) for *B. cinerea*, Dastur (1921) for infection with sporidia of *Tilletia Tritici* and Weber (1922) in the case of the corn rust, report that penetration occurred more frequently near the boundaries of epidermal cells than over the lumen of the cells. They consider that these places are more convenient for the exosmosis, from the content of the cell, of a chemotropical substance which would favor penetration. A second reason is that the easier way for a penetrating hypha to enter is through the middle lamella. Our observations on penetration by the black spot fungus show very little preference one way or the other.

The rose cuticle seems to be pierced wholly by mechanical means. The sudden enlargement of the infection peg into an infection hypha as soon as the cuticularized layers are reached, indicates that further penetration must take place in a different way in this region. We could not expect such a wide hypha to push its way through merely by mechanical action, although it is certainly flattened out considerably, which would suggest that it advances under pressure from the elastic cuticle. The lenticular pockets seen in the epidermis wall in cross sections of embedded material,

are usually greater in diameter than the hyphae which have formed them (text fig. 1 F, c, d, e). This indicates a chemical action of the fungus in making its way through the wall into which the enzyme produced has diffused a little beyond the boundary wall of the hypha. Ducomet (1907) claims that the regions around the penetration of the hyphae in the layers beneath the cuticle are the only ones in the host wall which take the cotton blue stain indicating therefore a change in the composition of the wall. The blackish cement that unites the parallel hyphae shows, according to him, traces of cellulose when colored with Congo red. He believes that the membrane is digested and that the intercalary substance is cellulose, chemically changed. We feel that the swellings of the wall so obvious in many cases at the places where haustoria will be sent through, indicate that an enzymatic action brings about the softening of the wall. Much swelling of the host wall is reported by Ward (1888) in the case of *Botrytis Tulipae* and by Blackman (1916) for *Botrytis cinerea*.

In the case of rusts especially, and of other fungi that form very fine haustorial threads, it is not improbable that penetration should take place merely by mechanical means. For *Diplocarpon Rosae* this might be the method by which a haustorium is sent into a cell from a hypha which is already intercellular (text fig. 1, C, E).

The first haustorium can be formed as early as fifteen hours after inoculation. Pole Evans (1907) reports that haustoria appear the third day from the uredo-mycelia of different rusts, and Higgins (1914) for *Cylindrosporium* on stone fruits observed them only after the fifth day. Twelve hours of favorable conditions are sufficient to insure infection and development of spots. The impervious cross-wall laid down by the fungus very soon after penetration of the cuticle may be a means of protecting the parasite against drying out and insuring its invasion regardless of later weather conditions.

The different types of hyphae developed by the fungus in the stages described are always intercellular (text fig. 1, F). The "intracellular" hyphae figured by Ducomet (1907) and mentioned by Wolf (1913) were evidently the "scouts" which are seen sometimes growing in the upper wall of the epidermis above the lumen of a cell, (pl. 20, figs. 30, c, and 32, a, b, c), giving the appearance as if they were intracellular. Cross sections of leaves show that scout hyphae are sometimes found in the lower layers of the outer wall, but even then they are separated from the lumen of the cell by one or two very thin layers (text fig. 1, F, at c and d). One finds intracellular hyphae in *Diplocarpon Rosae* only as a very late saprophytic growth of the fungus, when the stroma starts to form. As we know, this occurs late in the autumn when the leaves have fallen.

The collar formed around the base of the haustorium seems to be, in its time and manner of formation, very much like the collar described by Smith (1900) for the Erysiphaceae. It differs from it, however, in that its deposition never occurs in advance of the haustorium, but keeps pace with it until it is fully formed. This might indicate that in the case of *Diplo-carpon Rosae* the stimulating effect of the fungus extends only a short distance in advance of the haustorium.

All other investigators who have studied the collar and sheath of the haustorium agree, as we have already noted, that the formation of these inert parts of the haustorium is a result of the reaction of the host to the penetrating fungus. The question of proving the nature of these structures by using the proper stains or perhaps polarized light, remains open. It would be interesting to find out why the collar found is so often composed of two parts. Does this indicate that the host has ceased the deposition of the substance for a time and then has started, with renewed activity?

The collar at the base of the haustoria formed at the end of scout hyphae and from the cells of the synema are smaller than are the collars of the haustoria formed earlier. This might indicate that at the time when these are formed the attack on the leaf is so advanced that the cytoplasm has lost part of that reactive power which has an effect on the deposition of collar material.

Dufrénoy (1928, 1929, 1930) in presenting his observations on the influence of different parasites upon the cytoplasm of their hosts does not note any difference, as we have already seen, between the results in the behavior of obligate parasites and of facultative ones. It seems that different effects and more serious ones must be expected in the cytoplasm of a host cell which has been penetrated by a hypha of a facultative parasite which does not form any haustoria, than the effects produced by a haustorium which has invaginated only the cytoplasm.

Brown (1915) and Blackman and Welsford (1916) report that cells of bean pods inoculated with *Botrytis* are killed right after penetration of the cuticle. The same effect on the host is reported by Stevens and Hawkins (1917) for *Rhizopus*. By piercing the cells and killing the protoplast it produces the "leak" of strawberry. Hawkins and Harvey (1919) believe that *Pythium* punctures the cells of the potato tubers and then destroys their contents by secreted toxins. In the case of *Phytophthora infestans* on potato tuber it is reported by Jones, Giddings and Lutman (1912), that the host cells continue, for a short time only, to manufacture starch in the presence of the parasite; they die soon after they have been invaded. It might be also, according to these authors, that the attack is so sudden that the cell is unable to secrete the necessary diastases and is

caught with its chloroplasts full of starch.

Hartig (1882) considers as parasitic a number of Hymenomycetes which produce decomposition of the wood of living trees. In the case of *Septogloeum Hartigianum* (Hartig, 1892) on *Acer campestre* he reports that the mycelium is intercellular as well as intracellular and that haustoria are developed in the inner parenchymatous cells. On the other hand many cases are reported for strictly parasitic fungi where haustoria and cytoplasm of the host live for a long time without any sign of disturbance in the infected cell. De Bary (1887, p. 392) makes a very distinct difference between these two kinds. Ward (1902, 1905) when discussing the relations of rusts to their hosts says that they are different from those of a facultative parasite. They do not live upon the protoplasm of their host but consume only the food, and usually do not appear to injure the cells of the infected areas. Tischler (1912) in the case of *Euphorbia Cyparissias* attacked by *Uromyces Pisi* mentions that the haustorium in the host cell produces no disturbance for quite a long time. Allen (1923a, 1923b) reports the same behavior in the cells of Baart wheat which is susceptible to *Puccinia graminis Tritici* forms III and XIX. These are only a few of the many cases reported in the literature. Even among rusts, however, there are differences in the time when disturbance is first evident in the cell. Dodge (1934) reports that while cells of *Juniperus* penetrated by haustoria of *Gymnosporangium germinale* show very early disorganization, those of *Chamaecyparis* parasitized by *G. myricatum* are rejuvenated and have their life greatly prolonged.

In the case of *Diplocarpon Rosae* we have seen that browning of the cells penetrated by haustoria can occur at different times after infection. However, as yet, we do not know exactly how this browning and death of the cells occurs. Is it due merely to an accumulation of toxic substances or is it only a result of a premature drying out of the cells? A case comparable to this could be *Cylindrosporium* infecting stone fruits where Higgins (1914) reports that the death of cells is brought about by drying rather than by a toxic substance from the fungus protoplasm.

The fact that *Diplocarpon Rosae* forms haustoria in the rose leaf as early as fifteen hours after inoculation would seem to indicate that this fungus has reached a high degree of adaptation to parasitism. We know that in the case of many adapted parasites the haustoria keep on developing after their entrance and by branched, lobed or other complicated growths succeed in providing an extensive surface of contact between themselves and the host cytoplasm thereby increasing osmotic exchanges.

Haustroria of *Diplocarpon Rosae* do not develop much in surface before reaching maturity. We have seen also that a great number of them are

sent into the epidermis which has a poor supply of food. We are therefore inclined to consider that the fungus is capable of taking food also with the aid of the intercellular mycelium. The enzymatic action on host cellulose could break down this substance to a form which might be absorbable by the fungus. On the other hand, on the theory of structure of the cell wall advanced by Hansteen-Cranner (1922), there is a possibility that the intercellular hyphae might draw food by exosmosis from the cytoplasm. The same way of feeding must take place in the case of *Exoascus deformans* which according to Wallace and Whetzel (1910) develops subcuticularly and intercellularly but does not form haustoria. Considering these various views, one can say that *Diplocarpon Rosae* seems to be a transition form which has left the lowest step of parasitism but is still far from the level of a strictly obligate parasite.

SUMMARY

Inoculations of rose leaves with *Diplocarpon Rosae* show that the optimum conditions for infection are a saturated atmosphere and a medium temperature of about 75–80°F. In this case, spots appear on the third day. Petioles and young stems can also be infected. Of the varieties used, there is not much difference in the time necessary for the spots to appear. Infection is very little influenced by the absence of light.

Under optimum conditions, the spores placed on young leaves show complete germination in about nine hours after inoculation. By this time appressoria, if they are to be developed, will usually have formed. In the interval of 48 hours after inoculation, the fungus forms three kinds of hyphae which insure its spreading in healthy tissues and its further development in the intercellular spaces by three series of haustoria which penetrate the cells. A fourth kind of hyphal growth is developed from the parallel strands beginning about the sixth day after inoculation. These scout hyphae seek new places for penetration and formation of haustoria. The impervious cross wall laid down by the infection hypha by the thirteenth hour after inoculation, may be the means by which the parasite is protected against drying out and insures its invasion regardless of the weather conditions.

Penetration of the cuticle takes place mechanically. There is some evidence that the other layers of the wall may be modified by the chemical action of an enzyme secreted by the fungus so that they are more readily penetrated. Very likely, both chemical and mechanical action are necessary. Haustoria are surrounded by a collar-shaped basal mass which seems to be composed of material deposited by the host cytoplasm at the same time that the haustorium advances into the cell. No sheath was seen to

extend completely around the haustorium as reported for haustoria of many parasitic fungi. Browning of the host cells may start during the very early stages of invasion. This browning is mostly responsible for the dark color of the leaf spot.

Diplocarpon Rosae seems to represent a transition stage of adaptation to parasitic life. It has not as yet reached the level of a high type of parasitism.

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Explanation of plates

For a correct interpretation of the drawings see page 304 and 305 in the text.

The insets in some of the figures of plates 19 and 20 showing details in early stages of haustorium formation, are explained under "insets" at the end of the explanation of plates.

Plate 16

Artificially inoculated rose plant of the variety Red Radiance. The spots appeared the third day after inoculation. The photograph was taken two weeks later.

Plate 17

Fig. 1. Early phases in the germination of spores. About nine hours from inoculation. Shield-like thickenings indicate the places of germination.

Figs. 2 and 3. More advanced phases of germination; superficial germ tubes have formed appressoria. Shield-like thickenings and the rays surrounding the appressoria plainly visible. About ten hours old.

Fig. 4. Stages of germination, about ten to twelve hours old. No appressoria were formed at the end of germ tubes in these cases. The infection peg present.

Fig. 5. At a, a long superficial germ tube; at b, unusual germination.

Fig. 6. "Infection hypha" starts to form about thirteen hours after inoculation.

Figs. 7 and 9. Infection hypha penetrating in between two adjacent walls. Note the wall laid down across the hypha a short distance from the penetrating peg.

Fig. 8. Infection hypha in a thirteen hour old preparation, penetrating the outer wall of the epidermis.

Plate 18

Fig. 10. At a, unusually wide infection hypha; at b, the infection hypha has branched soon after penetration.

Figs. 11 and 12. "Fifteen hour" stages showing young haustoria with the deposited substance surrounding them.

Fig. 13. An older stage in the formation of the haustorium. The fungus thread has enlarged into a knob after escaping the collar-shaped deposit. Note the exaggerated swelling of the host wall.

Figs. 14 and 17. Mature haustoria found in fifteen-hour preparations. In fig. 14 the collar is shown composed of two parts, and in fig. 17 of only one part.

Figs. 15 and 16. Young haustoria, of the same age as the preceding, formed from an infection hypha which has penetrated directly through the outer wall.

Fig. 18. A case of penetration where, because of the position of the spore, the infection hypha has grown down directly between the two adjacent walls.

Fig. 19. Twenty-nine hour stage. At a, the first haustorium is formed directly from the infection hypha. A second haustorium, formed a little later and from a new portion of the initial intercellular hypha, appears lower down in the other cell.

Plate 19

Figs. 20-25 show another infection stage found in preparations from 36 to about 48 hours old. Some haustoria are indicated only by circles. The initial form of the end of the infection hypha is indicated.

Fig. 20. The branching of the infection hypha right above the place where the haustorium was sent in.

Figs. 21 and 22. More advanced stages of the same type of branching. In fig. 21, one branch has penetrated between two adjacent walls. At a and b the hand-like ends are ready to form parallel hyphae.

Fig. 23. Further development of the same stage. Haustorium formed at a. The branches at b and c are prepared to form haustoria also. At d, the branch will give rise to parallel hyphae.

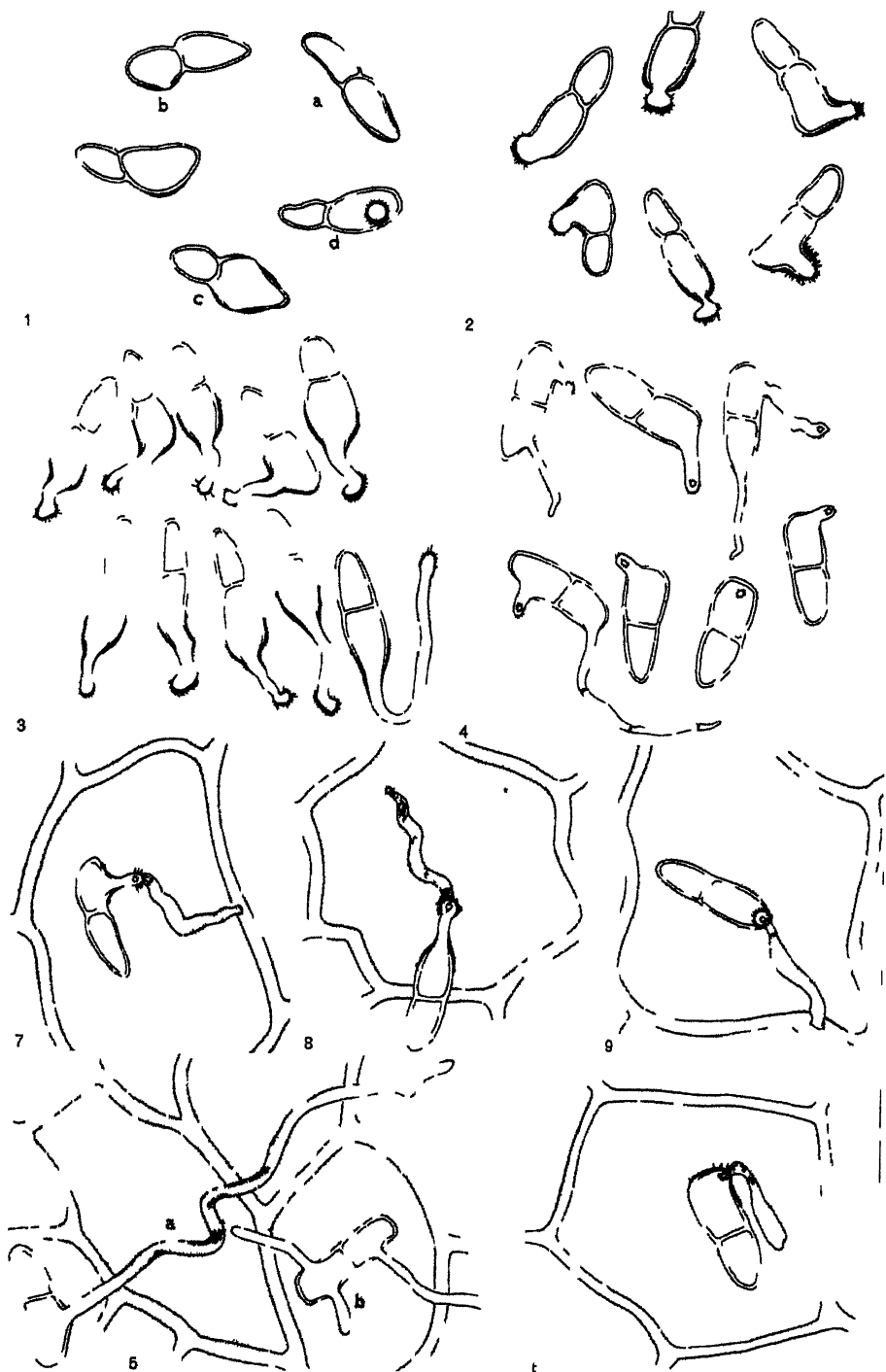
Fig. 24. The haustorium at a was formed at the initial infection. Haustoria at b and c were formed from the second type of branches the ones developed from the infection hypha just above the place where haustorium a was sent into the cell.

Fig. 25. 48 hour stage. Haustorium at a, formed probably by the initial infection. Note the hand shaped ends of hyphae which will form the parallel strands. At b, a little side branch which could form another hand-shaped growth. The finger-like projections at c, have already laid down cross walls.

Fig. 26. First cells of the "parallel hyphae." They are uninucleate.



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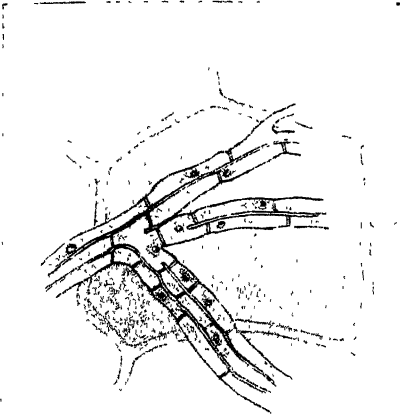


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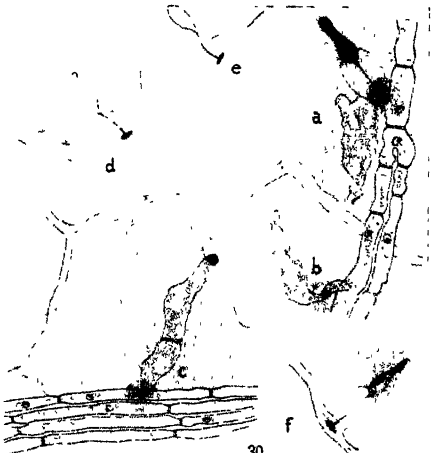
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Plate 20

Haustoria indicated in many cases only by circles.

Fig. 27. Active branching of parallel hyphae starting from a synema of three hyphae. Three days old.

Fig. 28. At a, a center where active branching has started. The figure shows different ways of association among the hyphae of different synemata. A few haustoria formed by the parallel hyphae are also shown. Three to five days old.

Fig. 29. The haustoria at a, b, c and d, were formed during earlier stages of infection. All the others originate from the parallel hyphae. Three to five days old.

Fig. 30. At a and b, formation of scout hyphae, by the branching out of the parallel hyphae, directly above the haustorium. At c the scout has penetrated between two adjacent walls. About six to seven days old.

Figs. 31 and 32 show numerous scouts which have formed the "fourth series" of haustoria. At a, b, c (fig. 32) the scouts are growing in the cuticularized layers above the lumen of the cell.

Explanation of "insets" of plates 19 and 20

Fig. 25. At d and e swelling of the wall at the place of penetration.

Fig. 21. At c and d young stages showing invagination of the plasma membrane of the host. Very little substance is deposited around the haustoria at c, d and e.

Fig. 22. At a and b, young haustoria surrounded by the deposited substance. The tips of haustoria enlarged into a knob.

Fig. 26. At b and c, the deposited substance has taken the shape of a collar. Haustoria are more enlarged at their tips.

Fig. 28. Young stages where the deposited substance is more abundant. At b, the collar-shaped deposit is composed of two parts.

Fig. 31. At b, more mature haustorium surrounded by the collar.

Fig. 30. At f, a mature haustorium. The haustorial thread has enlarged and elongated. At d, e and f are indicated some reactions of the haustorium affecting the surrounding host cytoplasm. (See text p. 316.)

INDEX TO AMERICAN BOTANICAL LITERATURE

1930-1934

The aim of this Index is to include all current botanical literature written by Americans, published in America, or based upon American material; the word America being used in the broadest sense.

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Meiosis in *Milesia marginalis*

S. M. PADY

(WITH PLATE 21)

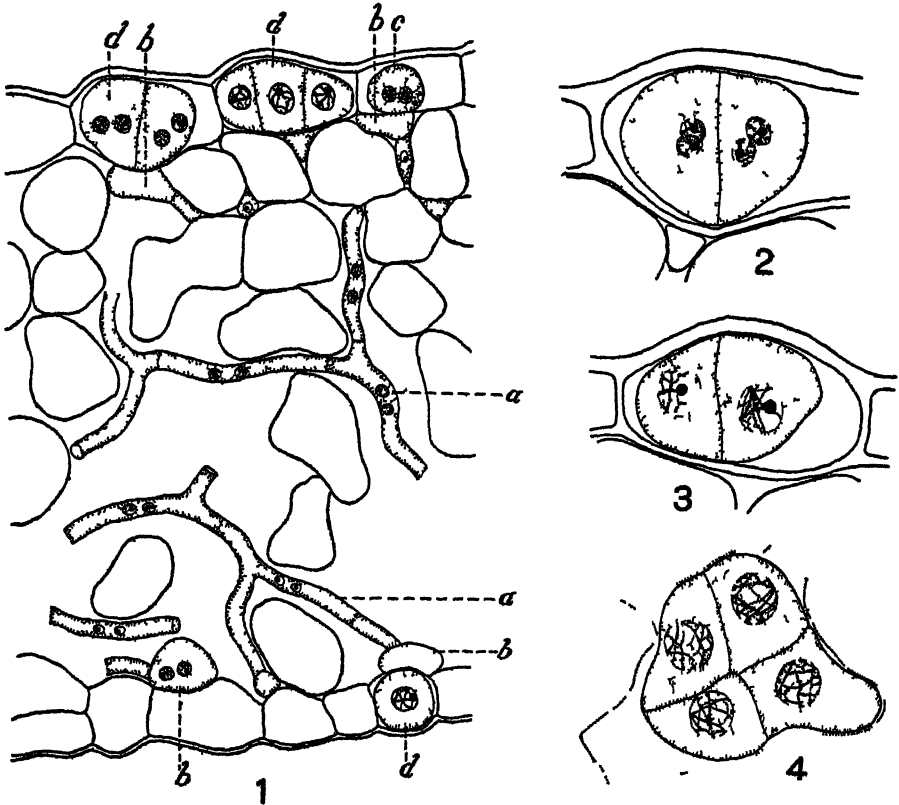
Nuclear division in the rusts has been the subject of study ever since the first nuclei were discovered by Schmitz in 1880, and valuable contributions have been made by Sappin-Trouffy, Blackman, Colley and many others. The rusts are rather favorable material for cytological study and present many and varied problems which are yet to be solved. I have recently reported on the two divisions of the fusion nucleus in the promycelium of *Hyalopsora aspidiotus* (Pady, *inedit.*), and shall give in this paper further data on the origin of the spindle, the details of prophase of the second division and the peculiar rod-and-ring division figure in the closely related species *Milesia marginalis*.

The teliospores of *M. marginalis* are formed in the spring in the epidermal cells of the old overwintered fronds of *Thelypteris marginalis*. The fronds usually die away as the season progresses but at the time the teliospores develop they are apparently still active. The development of the teliospores is indicated semi-diagrammatically in Text figure 1 which is a cross section through an overwintered frond. The mycelium, *a*, is composed of large hyphal cells, each with two prominent nuclei. Primordial cells, *b*, are formed just beneath both the upper and lower epidermis. The primordial cell penetrates the epidermal cell wall and its contents pass in to form the teliospore initial, *c*, which by growth and division becomes the multicellular teliospore, *d*. This type of development has been described in an earlier paper (Pady, 1933).

The two nuclei in the initial divide conjugately with the axes of their spindles parallel to the leaf surface. The daughter nuclei move apart and a cross wall is laid down, after which the nuclei take up a central position. This stage is well illustrated in text figure 2. Each nucleus has a well defined nucleolus and the chromatin appears to form a very coarse reticulum. When the young teliospore has reached its full size and nuclear division has been completed, the two nuclei in each cell come together and fuse. The nuclear membrane disappears at the point of contact and the two masses of chromatin merge. The two nucleoli remain distinct for a time, but later a single large nucleolus is present which presumably is the result of fusion, although the actual fusion has not been observed. The fusion nucleus rounds up and passes into a resting stage in which the chromatin is in the form of a very delicate but distinct network.

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The resting stage is of brief duration and the nucleus immediately enters into the heterotypic prophase. The chromatin comes to form a distinct spireme, and the nucleus increases tremendously in size. Text figure 3 is a cross section through a mature teliospore and shows clearly the two fusion nuclei. The chromatin is in the form of distinct threads which tend to lie around the periphery of the nucleus as is typical of diakinesis, with one or two strands connected with the nucleolus. In a surface view the



Figs 1-4 *Mlesia marginalis* Fig 1×920 Semi-diagrammatic drawing of a cross section of an infected frond illustrating the binucleate mycelium, *a*, in the mesophyll, the primordial cells, *b*, just below the epidermis, the initials, *c*, or young teliospores, the teliospores, *d*, in the upper and lower epidermis Figs 2-4×1540 Fig 2 Young binucleate teliospore Fig 3 Later stage showing fusion nuclei Fig. 4. Surface view of four-celled teliospore in upper epidermis

nuclei are very prominent (text fig 4), especially when stained with gentian-violet-iodine. The nuclei of the mature teliospore are thus not in the resting condition but have advanced into prophase. When germination takes place the fusion nucleus migrates out into the promycelium and immediately completes its division

When viewed from above the teliospores display a wide variation in shape, size and number of cells, and in these respects indicate clearly the modifying influence of the epidermal cells. In text figure 5 a number of teliospores have been drawn to illustrate the great diversity that may be found. Where the epidermal cell is narrow and elongated the cells of the spore may be in a linear row (text fig. 5, A, C, D). On the other hand where spatial relations require it, the teliospore may become more compact or spherical (text fig. 5, G, H, I, J). All gradations between these two types may be found (text fig. 5, B, E, F, J). In any group of spores there are

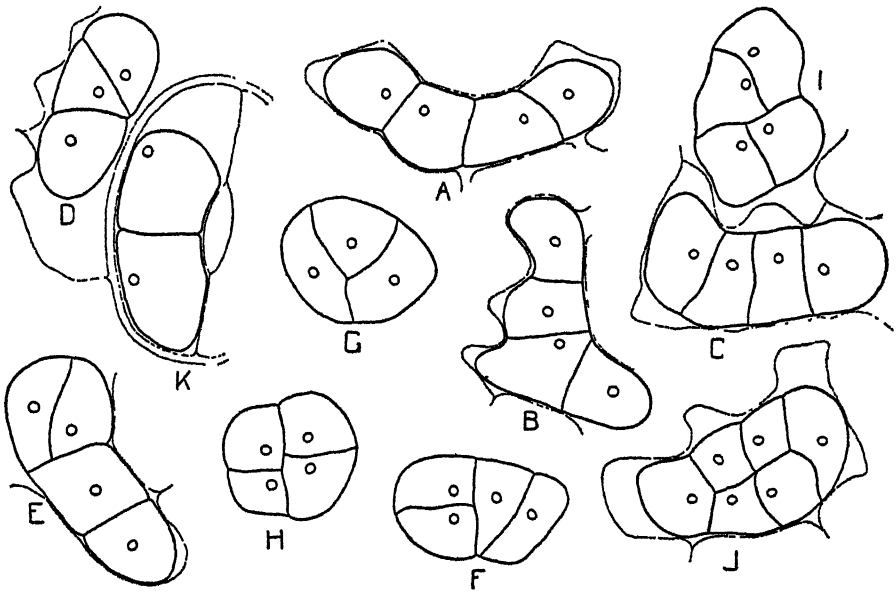


Fig. 5. A-J. Group of teliospores illustrating the variation in shape, size and number of cells and the modifying influence of the epidermal cells whose outline is shown by dotted lines. A single germ pore is found in each cell of the teliospore. $\times 1540$.

many irregularities, as for example the two-celled spore in the guard cell of a stoma (text fig. 5, K). Each cell possesses a well marked germ pore and it is at this point that the promycelium arises when germination takes place.

In *Milesia* germination follows as soon as the teliospores mature, and upon fulfilment of the essential conditions. As in *Hyalopsora*, moisture is the primary requisite and germination begins when the moisture content of the air approaches the saturation point. The material for this study was gathered on June 17th at 10:30 A.M. in the Temagami Forest Reserve in Northern Ontario. The fronds were placed between sheets of moist news-

print and kept in a moist chamber. Twenty-four hours later the fronds were examined under the dissecting microscope and the greyish white bloom on the surface of the leaves indicated abundant germination. By carefully selecting material under the high power of the dissecting microscope one is fairly certain of obtaining the desired stages.

The first indication of germination is a small white spot which appears in the cell wall immediately above the germ pore of the teliospore. This is the beginning of penetration of the wall and the formation of an opening through which the contents will pass into the promycelium. The opening, when completely formed, is slightly narrower at the base than at the top. Apparently penetration is by chemical action, and there is no sign of a papilla as in *Hyalospora*, where it appears to break the epidermis in an essentially mechanical way. It should be noted here that germination in *Hyalospora* takes place in the very young fronds and the walls of the epidermal cells are relatively thin, while in *Milesia* germination occurs in old overwintered fronds and the epidermal cell walls are greatly thickened. The many cells of a single teliospore do not germinate simultaneously and considerable variation may be found. It is not uncommon to find two promycelia from neighboring cells one of which is in a much more advanced stage than the other.

The cell contents now commence to pass through the opening and push up through the surface of the frond as a small sac or vesicle, which elongates to become the promycelium. While the promycelium is still small the large fusion nucleus moves toward the point of exit. In one case a nucleus was observed just as it was commencing to move through the opening. The advancing portion in the opening was level with the leaf surface, and the chromatin was in irregular masses throughout the body of the nucleus. In *Hyalospora* it was found that when the nucleus moved from the central part of the cell it lost its regular outline and stained very heavily, making it impossible to make out the details of its structure. As the nucleus of *Milesia* moves through the opening it becomes narrow and beaked and stains very heavily. Figure 1 (Pl. 21) shows an early stage. The nucleus becomes greatly attenuated and at one stage forms a dumbbell-like figure. Ultimately it slips through and remains for a time in the lower part of the young promycelium. At this stage the nucleus still tends to be quite irregular in shape and shows a strong affinity for the stain (Pl. 21, fig. 2). The cytoplasm remaining in the cell of the teliospore is markedly vacuolate (Pl. 21, fig. 1) but as the promycelium grows larger the entire contents move out leaving the empty cell wall behind.

The promycelia are easily dislodged and in the material studied were not readily found attached to the parent cell. This was undoubtedly due

to the necessity of repeated examination, although care was taken to reduce the amount of handling to a practical minimum. The rounded outline of many of the promycelia, together with the fact that the promycelium is independent of the teliospore in its subsequent development, seems to indicate that they may continue their normal development even when detached, provided that favorable conditions for development are maintained.

As the promycelium elongates the nucleus moves upward and assumes a more or less central position (Pl. 21, fig. 3). The nucleus is also at this stage usually somewhat irregular and stains very heavily, although the chromatin strands are sufficiently distinct to be recognized as late prophase. In the next stage the chromatin which is probably still in diakinesis, is more sharply defined and the nuclear membrane is distinctly rounded (Pl. 21, fig. 4). The nucleus now undergoes a marked change in appearance and the chromatin contracts into a heavily staining mass surrounded by a well defined hyaline area (Pl. 21, fig. 5). In the endosperm of *Iris*, Jungers (1931) found in late prophase that the nucleus contracted greatly and a perinuclear zone could be observed between the nuclear membrane and the cytoplasm. In figure 5 it is evident that no membrane surrounds the mass of chromatin and it is believed that it is represented by the rounded surface between the clear area and the cytoplasm. The spindle now begins to appear. The earliest stage I have found is shown in figure 6 in which the two poles of the spindle apparently coincide with the outer boundary of the clear area, suggesting very strongly the appearance of the intranuclear spindle, as found in the ascomycetes.

At each pole of the young spindle a small dark body is evident which is apparently a centrosome. From the poles the spindle fibres form a sharp V-shaped outline extending into the chromatin which stains very heavily. Although a sharply marked equatorial plate has not been observed, the chromosomes, which show up very clearly at this stage in *Hyalopsora*, are to be found at the centre of the spindle figure, and this stage undoubtedly corresponds to the equatorial plate stage. In figure 7 the chromosomes are beginning to move to the poles although individual chromosomes cannot be made out. This figure is very sharp and indicates the presence of a centrosome at each pole. The clear area around the spindle figure is less distinct in this figure, and in later stages it disappears entirely. Occasionally the chromosomes appear to move toward the poles in two distinct groups (Pl. 21, figs. 8, 9) but in general, anaphase is characterized by the spreading out of the chromosomes along the spindle figure (Pl. 21, figs. 10, 11). In figure 11 eight chromosomes may be counted. The chromosomes that have reached the poles begin to round up while the remainder are still

strung out along the spindle (Pl. 21, fig. 12). Telophase is characteristic with the two heavily staining somewhat irregular daughter nuclei at the poles, and the spindle fibres drawn into a single connecting strand (Pl. 21, fig. 13) which later disappears (Pl. 21, fig. 14). The presence of astral rays is indicated in the latter figure. They are very fine and delicate and the coarsely granular cytoplasm with its many small vacuoles makes their observation difficult.

During anaphase and telophase the division figure gradually elongates. This is clearly shown when figures 7 and 13 are compared. The young daughter nuclei also move toward opposite ends of the promycelia, and a nuclear membrane reorganizes about each one. A cross wall is formed and the promycelium becomes two-celled. The daughter nuclei immediately prepare for the second division, increasing greatly in size while the chromatin is in the form of a distinct reticulum (Pl. 21, fig. 15). As the nuclei enter prophase a well defined spireme is formed (Pl. 21, fig. 16), and a contraction phase similar to the one in the first division sets in (Pl. 21, fig. 17). The clear zone may appear on one side of the nucleus at first (Pl. 21, fig. 17) but it soon extends around the chromatin mass as contraction continues (Pl. 21, figs. 18, 19). In some cases a strand of chromatin may extend from the central mass through the clear area to the line bordering the cytoplasm (Pl. 21, fig. 19). This suggests that the nuclear membrane persists for a short time at least following the onset of contraction. In figure 20 two young spindle figures have been drawn, both taken from the same promycelium. At the tip of each pole is a minute but clear centrosome which appears to lie on the line previously occupied by the nuclear membrane. Similar spindles are shown in figure 21, only one pole of each spindle being visible. The spindle fibres extend into the central mass at a characteristic angle (Pl. 21, figs. 20, 21). In polar view their presence is indicated by a light area near the boundary of the heavily staining chromatin (Pl. 21, fig. 22).

It has not been possible thus far to demonstrate the origin of the two poles of the spindle. The evidence presented by the young spindles of both the first and the second divisions (figs. 6, 20) as to the existence of centrosomes at the poles of the spindles lying at the surface of the original nuclear membrane suggests that centrosomes are present on the nuclear membrane, and that they perhaps originate by division. Some of these figures are similar to those found by Olive (1908) in the multinucleate cells at the base of the aecium in *Puccinia Cirsii-lanceolati*, although they do not lend themselves readily to their interpretation as Hermann spindles.

In later stages the centrosomes at the poles are less distinct and the hyaline zone may persist for a time as in the first division (Pl. 21, fig. 23).

The spindle appears to elongate and the fibres form a rod-like structure (Pl. 21, figs. 24, 25). This may extend the length of the spindle to form a typical rod-and-ring type of division figure (Pl. 21, figs. 26, 27). These figures are similar in every way to those found in *Hyalopsora*. As anaphase begins the chromosomes move toward the poles (Pl. 21, fig. 28) and may become rather evenly distributed over the spindle (Pl. 21, fig. 29). The later stages are similar to those of the first division except that they are smaller (Pl. 21, figs. 30, 31). Occasionally promycelia are found in which division is more advanced in one cell than in the other, as shown in figure 32. In the upper cell is a typical telophase, while in the lower cell the spindle has practically disappeared and the young daughter nuclei are moving toward the opposite ends of the cell. This movement continues and the nuclei may come to rest against the median wall (Pl. 21, fig. 33). They are often pressed so tightly against the wall that they appear almost as a single nucleus. In the meantime the daughter nuclei have been gradually reorganizing and finally assume a spherical shape and a more median position in the cell (Pl. 21, fig. 34). A wall is formed and the promycelium becomes regularly four-celled. Each cell forms a sterigma (Pl. 21, fig. 35) and four basidiospores are formed as in other rusts.

DISCUSSION

In any group of promycelia there is a marked variation in size. Some promycelia are very small while others would be classed as "giants." In terms of volume their differences would be even greater. The explanation of this surprising variation, however, is not a difficult one. The teliospores of *M. marginalis*, as in related genera possessing intraepidermal teliospores, are extremely irregular. Cells of different teliospores and even cells of the same spore display differences in diameter and in volume. Since a single promycelium contains the entire contents of a teliospore cell, the variations in the size of the promycelia correspond to the variations found in the teliospores.

The two nuclear divisions in the promycelium of *M. marginalis* add considerably to our knowledge of division in the rusts and especially in the fern rusts. The details of prophase are similar to those described for *Hyalopsora* (Pady *inedit*) and agree in general with the accounts of Holden and Harper (1903), Blackman (1904), Colley (1918), Allen (1933) and others. The beginning of prophase in the teliospore is a characteristic feature of division in this rust. In rusts that germinate after a resting period, the fusion nucleus apparently overwinters in the resting condition. *Milesia* and *Hyalopsora* differ in that germination takes place without a protracted resting period, and the beginning of prophase in the spore is a device to

facilitate rapid germination. Moreover, development and germination take place early in the spring when the leaves of the alternate host, *Abies balsamea*, are in a susceptible condition. A peculiar anomaly is found in *M. fructuosa* which forms teliospores at the end of the current season. In one fixation made on September 15th the teliospores were mature and the fusion nuclei were all in advanced prophase. Subsequent development was not followed, but if germination follows, it takes place at a time when the alternate host is not susceptible. On the other hand it is hardly likely that these thin-walled spores would survive the long and severe winter of the region in which they were collected. Since there is the possibility that this species may be a variant of *M. intermedia*, further culture studies are desirable.

One of the peculiar features of late prophase in *Hyalopsora* was the tremendous reduction in volume of the fusion nucleus with the chromosomes formed inside what was called the nuclear membrane. In the light of the well marked contraction phase in *Milesia*, the presence of a contraction phase in *Hyalopsora* is strongly suggested. According to this view the so-called nucleus is not a true nucleus but is simply the contracted chromatic material in the late prophase or the equatorial plate stage. Minute centrosomes are found at the poles of the young spindle but their presence has not been demonstrated in later stages or in resting nuclei. These centrosomes are located on the nuclear membrane and when contraction begins, followed by disappearance of the nuclear membrane, they retain their position as indicated clearly when the spindle fibres begin to form.

At first the young spindle is entirely contained in the area of the original nucleus, so that in one sense it is intranuclear. Later, however, the spindle elongates greatly and the outline of the nucleus, which is represented by a clear area surrounding the group of chromosomes, gradually disappears. Olive (1908) found that nuclear division was intranuclear and the spindle as it elongated stretched the membrane considerably. Olive was able to differentiate between a Hermann "centralspindel" and mantle fibres. In *Milesia* such a distinction cannot be clearly drawn.

At this stage in both *Milesia* and *Hyalopsora* the spindle fibres form a cylindrical "rod" which, passing through the dense chromatin mass, gives the characteristic rod-and-ring appearance. The spindle fibres tend to retain the stain as B  lar (1926) found in the "achromatic cones" of the division figures in *Aggregata eberthi*, and thus accentuate this appearance. This type of division figure is prominent in the second division of the two fern rusts that have been studied and is probably characteristic of these so-called primitive rusts. *Milesia marginalis* and *Hyalopsora aspidiotus* are both long-cycled heteroecious rusts and exhibit none of the tendencies to-

ward reductions in their life-cycle, as for example, in *Puccinia Podophylli* (Jackson, 1931), or in their nuclear history, as in the various cytological races of the short-cycled rust, *Caeoma nitens* (Dodge, 1924). This would be expected when it is remembered that the rusts have evolved along with their hosts (Jackson, 1931), and thus these fern rusts in their evolutionary history have become fixed in their life cycle and in their nuclear history.

SUMMARY

1. The fusion nucleus in the teliospore, following a brief resting stage, enters into prophase forming a well defined spireme.
2. Germination is immediate and the nucleus enters the promycelium in late prophase.
3. A contraction phase is present in both divisions, the chromosomes contracting into a dense mass inside the nuclear membrane which gradually disappears.
4. The young spindle ends in centrosomes located in the region previously occupied by the nuclear membrane.
5. The chromosomes are more or less massed on the spindle and move to the poles in irregular masses.
6. A spireme is formed in the prophase of the second division.
7. Rod-and-ring division figures similar to those found in *Hyalospora* are characteristic of the second division and it is believed that they represent the equatorial plate stage.

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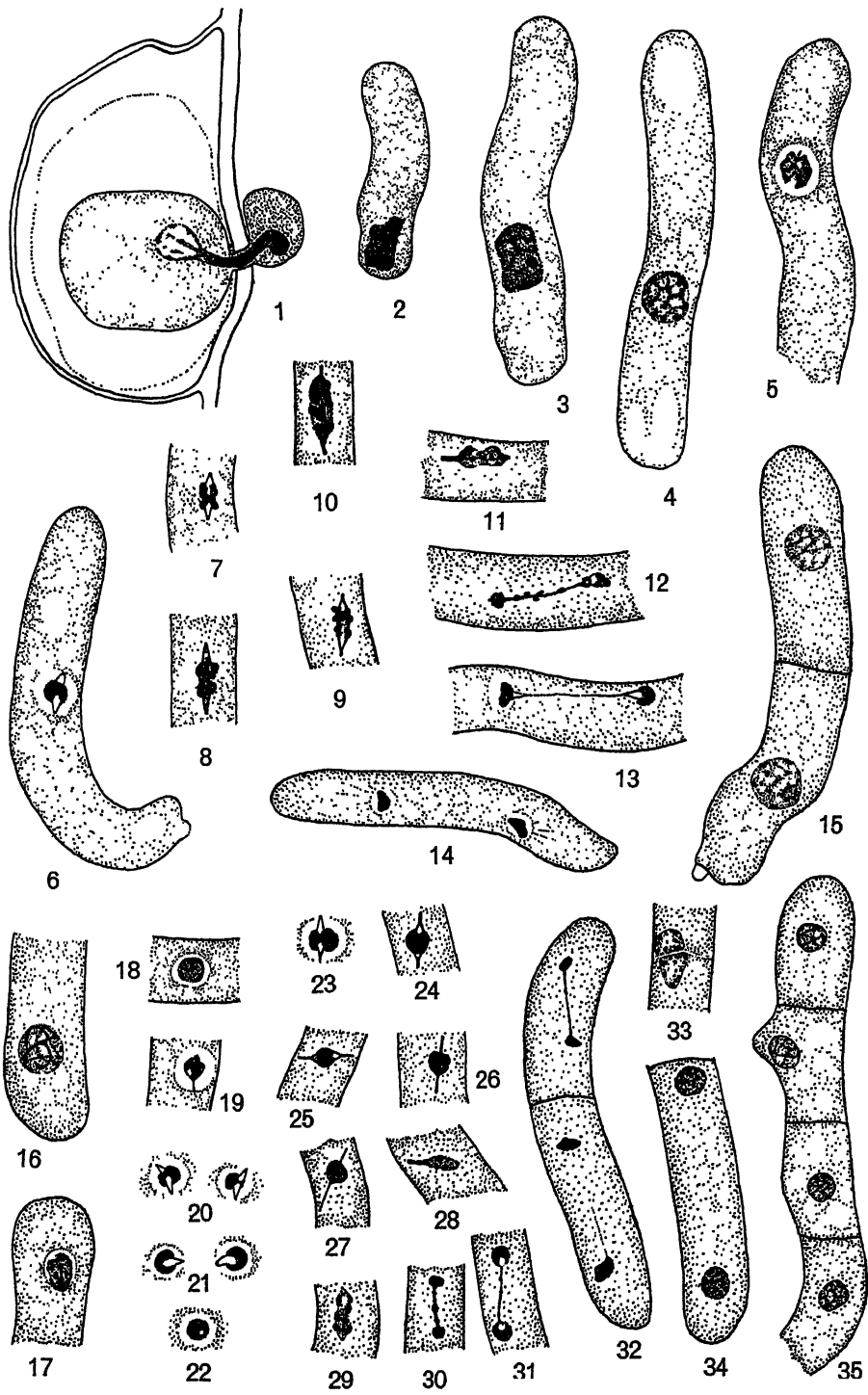
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Explanation of plate 21

All figures $\times 1540$

Milesia marginalis

- Fig. 1. Passage of nucleus into promycelium.
- Figs. 2-4. Late prophase.
- Fig. 5. Contraction phase.
- Fig. 6. Young spindle.
- Figs. 7-12. Anaphase.
- Figs. 13-14. Telophase.
- Fig. 15. Two-celled stage.
- Fig. 16. Prophase of second division.
- Figs. 17-19. Contraction phase of second division.
- Figs. 20-23. Young spindles.
- Figs. 24-25. Later stages.
- Figs. 26-27. Typical rod-and-ring division figures.
- Figs. 28-30. Anaphase.
- Figs. 31-32. Telophase.
- Fig. 33. Nuclei crowded against median wall.
- Fig. 34. Daughter nuclei completely reorganized.
- Fig. 35. Four-celled stage, two cells forming sterigmata.



The Origin of the foliar pseudo-bulbils in *Kalanchoë daigremontiana*

MARION A. JOHNSON

(WITH THREE TEXT FIGURES AND PLATE 22)

Bryophyllum has long been used by botanists as the classical example of a plant illustrating regeneration from detached leaves. Recent studies by Howe (1931), Naylor (1932), Yarbrough (1932) and Freeland (1933), have conclusively demonstrated that the plantlets in question may be produced, or are at least initiated, in the normal ontogeny of the leaf before removal and have thus cast serious doubt upon the advisability of regarding this phenomenon in *Bryophyllum* as regeneration. In this connection the writer wishes to call attention to *Kalanchoë daigremontiana* R. Hamet et Perrier de la Bâthie, which is far more prodigal in the production of foliar plantlets or pseudo-bulbils than is *Bryophyllum*.¹ Under good growing conditions a single plant may give rise to several hundred pseudo-bulbils; it is not uncommon for a large leaf to bear as many as sixty in various stages of maturity (figs. 1 and 2) while flowering specimens may produce a large number in the axils of bracts on the inflorescence axis.

Kalanchoë daigremontiana is native to Madagascar but has been widely introduced into the United States so that it may be readily obtained from growers of succulents. Greenhouse culture is similar to that for *Bryophyllum*.

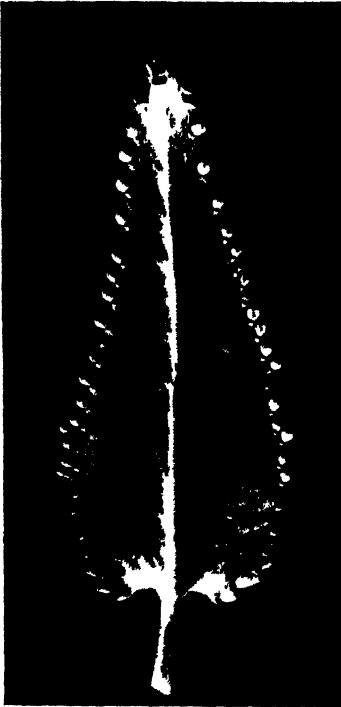
The habit of the plant resembles *Bryophyllum* in that it is relatively unbranched and about six decimeters in height although flowering specimens may measure two meters. The succulent leaves are opposite and decussate. The blade is broadly lanceolate with serrate edge and peltate base. The basal leaves are abscised until at the conclusion of the flowering period the plants are leafless. The inflorescence is a cyme bearing a profusion of pendulous, pink flowers, 2.5 cm. in length.

The pseudo-bulbils are produced freely in summer but are suppressed during December, January and February, except in the inflorescence. In March, however, when the days are appreciably longer they begin to appear once more on the leaves. They may also be induced at any time during the year from severed leaves either by placing them in moist chambers or by standing the petioles in water.

At the time of dispersal the pseudo-bulbils generally have six leaves. The first pair, somewhat unequal in size, are thick, sessile, and orbicular

¹ The ease with which *Kalanchoë daigremontiana* may be propagated by pseudo-bulbils has recently been noted by Swingle, C. F. 1933. The easiest plant in the world to propagate. Jour. of Heredity, 25: 73-74.

in shape with a broadly obtuse tip and entire edge. The second pair are broadly elliptical, with obtuse tip, entire edge and with the base tapering into a short petiole. The third pair is similar to the second except that the blade is more elliptical in shape and has two to four deep serrations on each edge. The base of the pseudo-bulbil is much swollen immediately above the attachment to the parent leaf. Roots arise from this region as well as from both the nodes and internodes and may make their appearance before the plantlets are detached.



Figs. 1 and 2. Fig. 1. Photograph of leaf from lower surface showing pseudo-bulbils; $\times \frac{1}{2}$. Fig. 2, lower surface of leaf showing pseudo-bulbils bearing plantlets of a second order; $\times 1$. Both leaves were in condition shown when removed from plant for photographing.

The material for this study was taken from leaves of well grown plants. The terms pseudo-bulbil and plantlet have been used interchangeably although it is recognized that technically pseudo-bulbil may be considered the more appropriate because of the enlarged, bulb-like base. On the other hand, the term plantlet may be equally well employed since roots, stem and leaves are organized before dispersal.

DEVELOPMENT OF THE LEAF

In order to determine the ultimate origin of the pseudo-bulbils it was necessary to work out the ontogeny of the leaf. The following brief outline is presented to clear up those points having a bearing upon the problem. The primordia appear decussately as broad, round-topped cones of meristematic tissue from the stem tip. When an approximate height of 90μ is reached, the conical alignment is displaced by considerable enlargement of the cells in the basal portion of the abaxial faces so that the primordia curve inward until they completely overarch the apex of the stem.

Cell division is quite general throughout the primordia at this stage, three chief centers of activity can, however, be recognized: the first is at the tip and is responsible for apical growth which persists for a short time; the second is in the region of the midrib and contributes to that part of the blade and petiole; while the third is located in the adaxial face and gives rise to the lamina.

Apical growth ceases when the primordia are about 0.9 mm. in length. Meristematic activity is now transferred from the distal end to the base in the form of two ridges of tissue extending along the edges of the adaxial face. These curve inward until they meet, thereby forming an arc of tissue which is responsible for the turned up edge of the peltate base seen in adult leaves.

Transverse sections through young leaves show eleven layers of cells (fig. 4). The nine inner ones give rise to the mesophyll and can be traced to the activity of a single sub-epidermal cell at the edge of the leaf. This cell is but one of a row extending throughout each ridge of the developing lamina. These mesophyll mother cells, as they may be designated, maintain their position according to the scheme described for the tobacco leaf by Avery (1933). The mother cell divides at right angles to the upper surface of the leaf, then the outer cell divides parallel to the leaf surface forming the two cells *a* and *b* seen in fig. 4. One of these, say the upper *a*, becomes the initial which divides as the original mother cell until three cells equivalent to the ones just described are formed; but now, it is the lower instead of the upper cell from this new series which becomes the initial. Thus it can be seen that the position of the row of mesophyll mother cells remains relatively unchanged throughout the development of the leaf. The young lamina is built up with an entire edge until a length of about .9 mm. is reached, when slight undulations make their appearance. These are pre-determined by a difference in the rate of division which exists in certain portions of the row of mesophyll initials and its derivatives. Ultimately all the cells in the active centers mature forming conspicuous serrations, while

the initials at the base of the notches remain meristematic. Cell division here is retarded although not completely suspended, as is evidenced by the occurrence of occasional mitotic figures.

The structure of the mature leaf is extremely simple, there being no differentiation into palisade and spongy mesophyll. All of the cells except those in the epidermis are well supplied with chloroplasts. Mature leaves, exclusive of the midrib, are from 15 to 20 cells in thickness. A bluish-red pigment which has been identified as anthocyan is found in the second layer of cells within the epidermis (*c*, fig. 4), as well as in localized areas throughout the leaves.

ORIGIN AND DEVELOPMENT OF THE PSEUDO-BULBILS

The production of the pseudo-bulbils is intimately associated with the origin of the notches between the serrations and in time of development parallels the growth of the leaf. The inhibitory action of rapidly growing plantlets on the growth from other notches as described by Loeb (1924) for *Bryophyllum calycinum* Salisb. could not be detected in attached leaves. As has been noted maturation of the leaf begins first at the distal end and progresses basipetally in an orderly sequence, thus the oldest plantlets are found at the tip with a graduated series of younger ones extending down into the notches of the peltate base. All stages in development may be had in leaves between five and twenty centimeters in length.

The youngest leaves in which the origin of the pseudo-bulbils could be traced were from 2.5 mm. to 3 mm. in length. Figure 5 was drawn from a section taken through the bottom of a notch from such a leaf and when compared with figure 4, which represents a comparable view before a new plantlet has been initiated, indicates the extent to which development has taken place. It is to be noted that the row of mesophyll initials can no longer be distinguished. In their stead six rows of subepidermal cells (seen between *a* and *b* in fig. 5) two tiers in depth extend along the bottom of the shallow notch so that the edge is considerably thickened. These cells are smaller than those from a similar position in younger leaves and continue further around the edge (between *b* and *c* in fig. 5). Serial sections through older notches show that the six rows of cells just described initiate the formation of a very active meristem which is oval in shape, 84μ in length by 68μ in width and 4 to 5 cells in depth with a convex outer surface covered by a thick cuticle (figs. 6 and 8). This meristem becomes more massive until its free face measures some 112μ by 109μ when two leaf primordia originate in the same manner as from the stem tip of an older plant except that one can be recognized slightly earlier and is the larger of the two (fig. 10). The less active cells in the slight concavity between the primordia

represent the stem tip. A prominent provascular strand soon differentiates from the base of each primordium until it connects with the veins entering the notch.

Coincident with the organization of the plantlet meristem the cells seen in the region between *b* and *c* in figure 4 become active and extend the bottom of the notch over the edge of the leaf as a trough with the tip curved upward (figs. 6, 7 and 13). For want of a better name the writer has designated this projection as the "peg." It matures at the tip and is then built out from the base so that the plantlet is carried out of the notch. At maturity the peg is 3 to 4 mm. in length and is inclined from 60°-120° to the plane of the blade.

The pronounced swelling at the base of the first pair of leaves where attachment to the peg is made appears early and in figure 10 is seen as a slight protuberance on the right of the large primordium. It is formed by the ultimate enlargement and change in orientation of the cells in this region and not by an appreciable increase in cell division as might be expected. Plantlets about .5 mm. in height show that approximately seven rows of cells in the outer part of the base are involved. At first these cells are uniform in size and arranged vertically, however, as growth progresses some six or eight cells from each row commence to enlarge almost at right angles to the leaf primordium while those slightly lower in the same rows elongate parallel to the same axis. The result of this unequal growth is a large swelling with a sharp constriction where attachment is made to the peg (figs. 3, 12 and 14). The cells in the attachment are somewhat bent back on themselves and appear to be under stress, perhaps due to tension set up by the cells in the swollen region above. The enlarged base of the pseudo-bulbils which may measure 1-2 mm. in diameter, at time of shedding, serves a threefold function: first as a storage organ; second as a point of origin for the first roots; and third as a unique organ for detaching the plantlets from the peg.

The storage function is first manifest in the peg, where the parenchyma becomes filled with large starch grains. This reserve food supply gradually diminishes as the tissues of the pseudo-bulbil mature and the cells in the swollen base and thickened leaves soon contain an abundance of starch which is confined to the peripheral layer of the cytoplasm. The prominent central vacuole serves as a reservoir for water, thus the young plantlet is provided with a food and water supply until it can establish itself. A well developed cuticle aids in reducing transpiration.

The appearance of the root is late in comparison to that of the stem and leaf primordia; pseudo-bulbils with the first pair of leaves 3-4 mm. in length show nothing which can be interpreted as root development (fig.

14). The writer's observations thus far do not include the exact stage at which the roots are initiated, but plantlets with two pairs of leaves have well organized roots as are to be seen in figure 3. Each large root is accompanied by two lateral ones, so that they occur in groups of three on a broad base of meristematic tissue which is intimately associated with the vascular system. Roots may be produced from both the nodes and the internodes as well as the swollen base of the plantlets.

The problem of pseudo-bulbil detachment has proven to be of considerable interest. The presence of dozens of plantlets under well grown specimens in the greenhouse would suggest that they might be cut off by abscission layers as is common in deciduous leaves. Examination of the

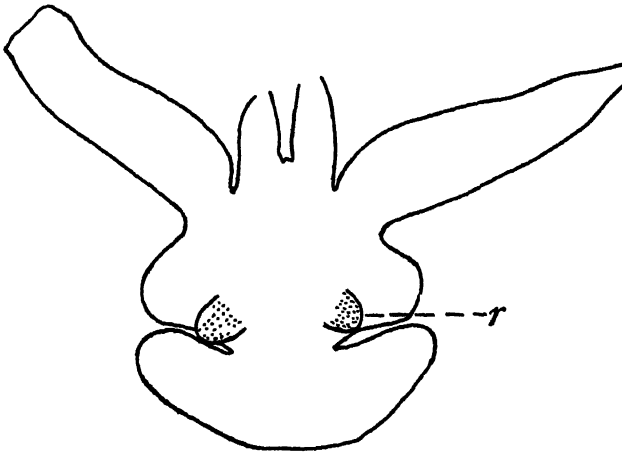


Fig. 3. Transverse section through peg showing: attachment of pseudo-bulbil; swollen base which serves as a fulcrum in detaching plantlet; *r*, root; $\times 24$.

attachment region fails to show any sign of an abscission layer. Cytologically the cells have every appearance of being in good living condition. They are distended by a large central vacuole, have thin walls without visible signs of disintegration and resemble the other living cells except in being free from starch. Evidence to support the usual explanation of abscission is lacking. A study of sections as represented in figure 3 suggests that pressure brought against the peg by the developing roots might break the attachment; while this may be a contributing factor, yet roots often appear while the plantlets are still attached to the parent leaf without freeing them (fig. 2). The method of detachment appears, in final analysis, to be mechanical and is interpreted as follows. In figures 1 and 14 it will be seen that the pseudo-bulbils are offset by the peg so that they are not protected by the edge of the leaf, and therefore may be readily struck by rain-drops or brushed by swaying leaves. Furthermore the plantlet appears to

have been girdled by an invisible band which has almost effected separation from the peg (fig. 3). The attachment is small in comparison with the swollen base of the plantlet although it is traversed by two or more vascular bundles. It is to be further noted that the enlarged base is asymmetrical with the outer face the more highly developed. A comparison of the plantlet to a lever of the first kind attached at one end near which there is a fulcrum is especially enlightening. The connection between plantlet and peg represents the attachment of the lever, while the stem axis and leaves comprises the lever itself and the projecting edge of the asymmetrical base is in contact with the peg which serves as the all-important fulcrum. It can readily be seen that sufficient force applied to the lever will detach the whole system. This is to be expected since the vascular bundles of the slender attachment are small and the remaining cells are thin-walled and distended by turgor pressure in addition to being under tension, all of which contributes to the ease with which they may be severed by lateral stress. The efficiency of this mechanism is easily demonstrated, a slight touch on the thick stiff leaves of the pseudo-bulbil frees it at once while greater force is required when applied at right angles to the peg in pulling off the plantlet. In undisturbed specimens plantlets of the first order remain attached to the parent leaf until they in turn bear pseudo-bulbils of a second order, which certainly is proof that an abscission layer has not interrupted the vascular supply (fig. 2). Furthermore, in the greenhouse withered leaves are frequently found on old plants with a number of the plantlets still attached which are finally shed with the old leaves. There is little doubt but that rain and wind are sufficient to detach the plantlets in nature. The importance of rain as a disseminating agent is further suggested by the fact that in the native habitat pseudo-bulbil formation is initiated during the rainy season. The nicety with which such a system functions in vegetative reproduction is readily appreciated for plantlets are not likely to be detached until they are large enough to offer resistance to mechanical force and by that time they are stored with starch and water and have leaves, stem and roots well organized.

DISCUSSION

The origin and organization of the meristems from which the pseudo-bulbils in *Kalanchoë daigremontiana* are produced compare favorably with that reported for the development of the foliar embryos of *Bryophyllum calycinum*. In both cases the development is directly associated with the normal ontogeny of the leaf and can be detected when the leaves are from two to four millimeters in length. This activity in *K. daigremontiana* is

initiated in the row of mesophyll mother cells located in the bottom of the young leaf notches. The same condition exists in *Bryophyllum calycinum* judging from the drawings of Naylor (1932) and Yarbrough (1932). The chief difference between the two is in the time of root development which is considerably later in *Kalanchoë daigremontiana*. In the plantlets observed no sign of a root meristem was found until after the first pair of leaves were partially developed.

Two species of *Kalanchoë*, *K. daigremontiana* and *K. tubiflora*, so far as the writer is aware, are unique among plants in having their pseudo-bulbils seated upon a peg from which they are readily detached by mechanical means. Advantageous as the peg may be for dispersal, it is not always developed, for example in the former species it is absent from the plantlets formed in the inflorescence. It is to be noted that here the plantlets are more exposed to the action of wind and rain which may free them more readily than from the leaf. This, however, is not being advanced as a factor in suppressing the peg. The swollen base is developed as in the foliar plantlets. Dispersal in this case may be delayed until the death of the parent plant which under greenhouse conditions appears to occur soon after flowering. When severed leaves are placed on moist soil, the plantlets produced root freely, but again, the peg is poorly if at all developed. In this instance there is no method for dispersal except through the decay of the parent leaf and subsequent washing by water as is reported by Ridley (1930) for *Bryophyllum calycinum*.

The detachment of the pseudo-bulbils by mechanical means as seen in *Kalanchoë daigremontiana* appears to be similar to that in *Cystopteris bulbifera* (L.) Bernh., *Lycopodium Selago* L., and *L. lucidulum* Michx. The propagules in these instances are supplied either with a swollen base or thick sessile leaves and a fragile attachment which is easily pried loose. In the case of *L. lucidulum* Smith (1920) reports that the bulbils are freed in part by a disorganization of the xylem walls in the attachment. Nothing resembling stages in decomposition of cell wall or cell contents could be found in *K. daigremontiana*. Smith (1920) further describes the accumulation of starch in *L. lucidulum* as being due to the absence of phloem in the constricted attachment. This raises the question as to whether the food stored in the pseudo-bulbil was produced by the parent leaf or the leaves of the pseudo-bulbil itself. The first evidence of stored starch is to be found in the peg and could have come only from the parent leaf since the pseudo-bulbil is still embryonic. Later this store of starch is gradually depleted and when the plantlet has developed its first pair of leaves, starch appears in the swollen base. The differentiation in the vascular bundles of the attachment is not sufficient to detect the presence of phloem with

certainly so that the reserve starch for the young plantlet may have come from either the parent leaf, from its own leaves or perhaps from both.

The view held by Kupfer (1907), Yarbrough (1932), and others, that growth from preformed meristems is not to be included under the phenomenon of regeneration is supported and strengthened by the writer's observations on the production of foliar pseudo-bulbils in *Kalanchoë daigremontiana*. It is to be noted that in summer these plantlets are not only initiated but are developed without a period of dormancy. They appear so early in the ontogeny of the leaf that they may be seen with the unaided eye long before the leaf has reached maturity. Furthermore they are not organized from mature cells which have been regenerated or rejuvenated but from cells which have never lost their meristematic qualities. It is clear then, that the formation of these plantlets represents a phase of growth intimately associated with the development of the leaf and which results in an efficient means of vegetative reproduction. The cessation of growth in the plantlet meristems during the winter months brings about a condition similar to that generally seen in the foliar embryos in *Bryophyllum calycinum* under greenhouse conditions. According to the observations of Ridley (1930) this species when growing in Jamaica bears bulbils on its leaves in abundance. If these observations by Ridley or the abundant production of pseudo-bulbils in *K. daigremontiana* had been available to Loeb (1924) it is doubtful whether he would have advanced his explanations for the activation of the foliar meristems in *Bryophyllum calycinum*.

The problem of inducing and breaking dormancy in plants may well be attacked in *Kalanchoë daigremontiana* which is more favorable for this type of experimental work than *Bryophyllum calycinum* upon which recent studies have been made. A distinct advantage over *Bryophyllum* is that plantlets are produced in abundance during the summer under good growing conditions but their growth is retarded throughout the winter so that dormant meristems are to be found in the leaf notches. These, however, become active upon the return of summer and grow out into plantlets. Experimental results obtained by varying the length of day, quality of light, humidity, amount of water, etc., either during summer or winter could be easily measured against the controls which according to the writer's experience have a definite, clear-cut behavior depending upon the season. Since plantlet formation is restricted to the time of year having long days it may well be that length of day is a factor in inducing or breaking dormancy of the foliar meristems. Experiments in support of this supposition have not been attempted. The failure of *Bryophyllum* to produce plantlets freely with the leaves attached may in part account for the negative results obtained by Freeland (1933) upon varying the length of day,

humidity and water supply for young plants. *Kalanchoë daigremontiana* is also suitable for studies in the activation of the dormant foliar meristems on severed leaves. Furthermore, the mechanism for detaching the plantlets is ideal for obtaining more accurate data on the mass relation of regeneration as demonstrated by Loeb (1924) for *Bryophyllum calycinum* leaves.

SUMMARY

1. A noteworthy feature in the life-cycle of *Kalanchoë daigremontiana* is the production of pseudo-bulbils in the leaf notches of attached leaves. The degree of development attained is dependent upon the season. During the summer the pseudo-bulbils are matured and detached from the parent leaf while in winter they remain dormant as embryonic tissue in the leaf notches. Dormancy may be broken, however, by severing the leaves and placing them under suitable growing conditions. This treatment yields vigorous plantlets in a short time.

2. The ontogeny of the leaf is described briefly.

3. The origin of the pseudo-bulbils is determined. They are developed from the row of lamina mother-cells in the bottom of the leaf notches during the ontogeny of the leaf. The earliest stages are found in leaves 2.5 to 3 millimeters in length. Evidence is presented which indicates that the production of the pseudo-bulbils is not to be regarded as the result of regeneration.

4. The pseudo-bulbils are seated upon a grooved peg which is developed from the bottom of the leaf notches. This appears to be a unique organ in plants.

5. The pseudo-bulbils are provided with a swollen base which serves as a storage organ for water and food materials. It also acts as a fulcrum against which force supplied by wind, raindrops, etc., pries the plantlets from the peg. An abscission layer is not formed.

6. It is suggested the *Kalanchoë daigremontiana* would be a suitable plant for physiological studies in inducing and breaking dormancy.

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Explanation of plate 22

Fig. 4. Transverse section through young leaf showing: mesophyll mother-cell which has recently divided forming the two cells *a* and *b*; *c*, layer of cells containing anthocyan; $\times 365$.

Fig. 5. Transverse section of leaf cut through notch showing: early stage in organization of pseudo-bulbil meristem from cells opposite *a*, these cells are derivatives from several mesophyll mother-cells similar to that in fig. 5; the cells in region between *b* and *c* which develop the peg; $\times 365$.

Figs. 6 and 8. Transverse sections through leaves showing continuation of growth initiated in fig. 5; *p*, peg; $\times 365$.

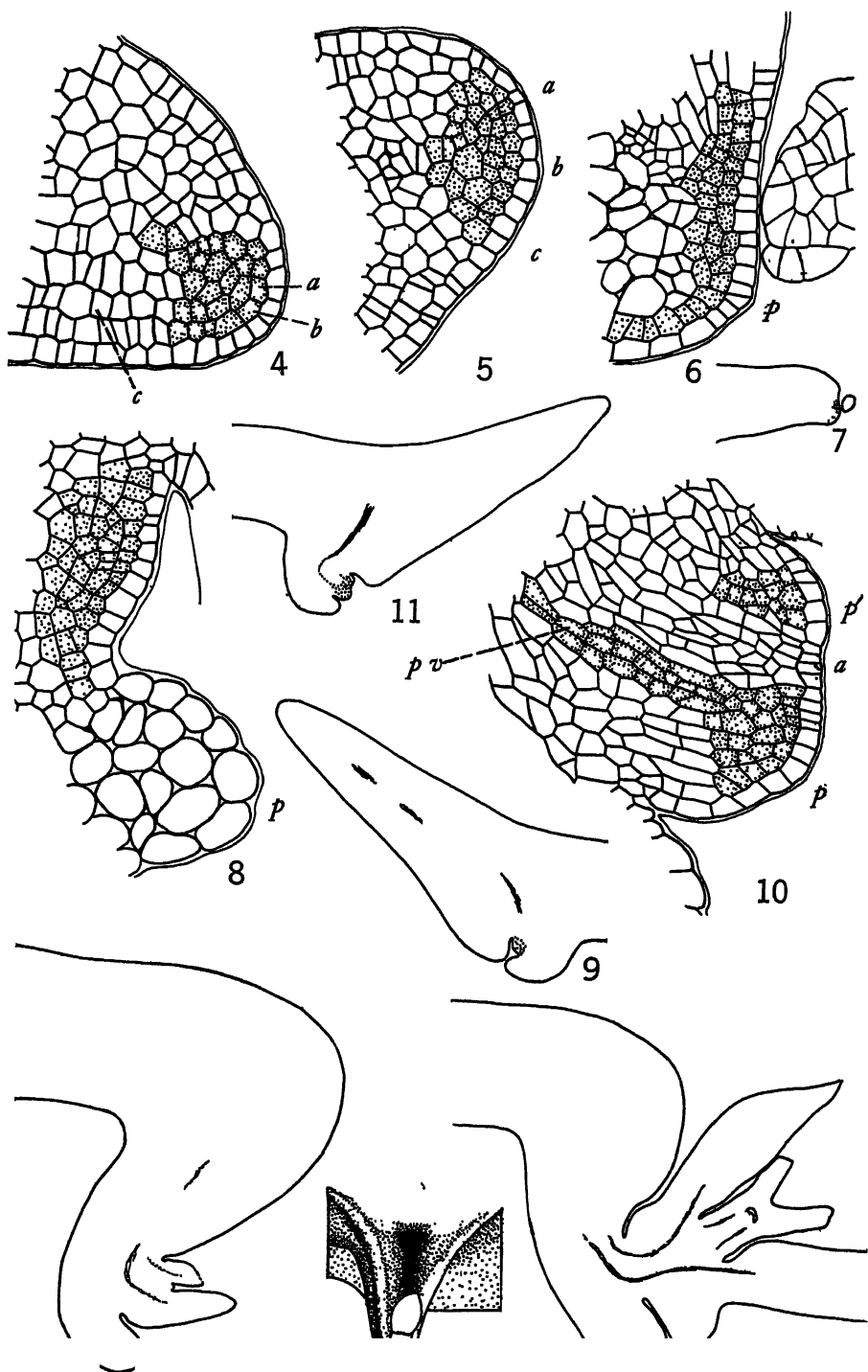
Figs. 7 and 9. Transverse sections through leaves showing portions from which figs. 6 and 8 were drawn; $\times 35$.

Fig. 10. Section comparable to fig. 7 but further developed showing: organization of young pseudo-bulbil; *p*, primordium of first leaf; *p'*, primordium of second leaf; *a*, apical meristem of plantlet; *p v*, provascular strand; $\times 365$.

Fig. 11. Transverse section through leaf showing position of plantlet shown in fig. 10; $\times 35$.

Figs. 12 and 14. Transverse section through leaves showing further development of pseudo-bulbil. Fig. 12, $\times 35$. Fig. 14, $\times 16$.

Fig. 13. Diagram showing grooved peg with pseudo-bulbil from above; $\times 10$.



A new Brazilian species of the genus *Utricularia*

EDMUND M. MERL

(WITH TWO TEXT-FIGURES)

In his paper on the variety of structure and function in the traps of *Utricularia* and *Polypompholyx*, F. E. Lloyd¹ describes among others the bladders or traps of a yet undescribed Brazilian species which, on the basis of the material supplied to me by Professor Lloyd, I regard as a new species, which I have named *Utricularia Lloydii* (Merl) in his honor.

Since we are concerned with a species interesting from various points of view, I shall present in what follows a more intimate description.

U. Lloydii occurs in the region of the Rio Branco² but on account of its small size is easily overlooked. The inflorescence, at most 7 cm. in height, (fig. 1) bears only one yellow flower, aside from which there is however an additional flower bud which appears usually not to develop further.

At the base of the scape there arises in its uppermore zone a number of rhizoids with short, lateral, adhesive shoots, densely clothed with sessile, knob-shaped, glandular hairs (lower pointer, fig. 1). Just below there extend stolons which carry leaves, bladders and additional secondary stolons. These secondary organs which arise from the primary stolon occur on its flanks and are lateral but nevertheless approach each other somewhat dorsally.

The apex of the stolon is straight—not, as in many other species, in-rolled crosier fashion. The narrow linear leaves usually bear two to three bladders. The upper ones are usually displaced into the leaf lamina and placed at right angles to this, their openings being turned toward the apex of the leaf.

The species is particularly worthy of note, however, because of the occurrence of bladders of two sorts. (fig. 2). This peculiarity, to which Lloyd and others already have drawn attention, is shared by other representatives of the genus.³ Nevertheless, the phenomenon is limited to only a few species. For the sake of completeness, I shall here repeat a number of details already pointed out by Lloyd. The bladders of *U. Lloydii* are extremely short-stalked, indeed almost sessile. The stolon bladders (fig. 2a) are in general of different form from those on the leaves (fig. 2b).

¹ Lloyd, F. E. 1932. The range of structural and functional variety in the traps of *Utricularia* and *Polypompholyx*. *Flora* 126: 303–328.

² The collector's label reads: Fl. amarela; plantinha dos terrenos arenosos e humidos. Retiro do Serra da Lira, Rio Branco. Colhido por J. Geraldo.

³ Compare, for example, *U. volubilis* R.Br. (Merl, 1915).

The former have on their front aspect, above the entrance, two short, broad, approximately triangular antennae which bear trichomes with an elongated basal and a rather long end cell, arranged in rows. Additional rows of similarly formed trichomes extend laterally to the entrance and stand in especially dense order on a chin- or ramp-like projecting portion of the bladder beneath the entrance, occupying almost the whole lower half of the anterior part of the bladder. Similar but shorter trichomes occur



Fig 1. *U. Lloydii* Merl. An entire plant enlarged about one-third. Above, left the basal portion of another plant. The upper pointer indicates the seed-shell, the lower a stolon with short, fusiform branches. Above, right other flowers and a pair of sepals.

also in the entrance on the anterior portion of the door, which is free of these in its innermore region.

Very different from the above are the bladders which occur on the leaves. These possess thinner, longer, and somewhat downwardly bent, horn-like antennae, on which there occur a number of merely sessile, spherical, glandular trichomes, such as occur on other parts of the plant,

for example on the lateral outer walls of the bladder, adhesive shoots, stolons, etc

There is an especially strong covering of these sessile trichomes clothing the ramp. In the upper part of the entrance we find short-stalked trichomes with more elongated end cells, but in the middle of the door there is a single trichome with an enlarged basal cell and a bristle-like elongated end cell. In both kinds of bladders there occur immediately under the

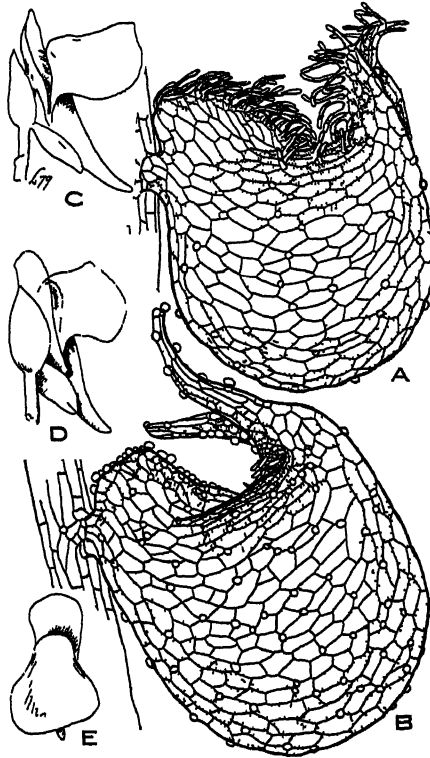


Fig 2 a, bladder usually found on the stolons, b, bladder usually found on the leaves, c-e, flower in three positions

threshold trichomes with a club-shaped end cell, following which there is a group of two-armed hairs with similar end cells, such as occur scattered on the entire inner wall surface of the bladders.

In the matter of the distribution of these two kinds of bladders there are frequent exceptions, so that where one would expect one type of bladder to occur one finds the other kind

Since the form of the bladder is an important, if only recently appreciated character, useful in the identification of *Utricularia* species, it wil-

be interesting to note in what other Brazilian species we find analogous conditions to those above described.

The type of bladder with broad antennae and external files of trichomes occurs chiefly among the violet or blue flowered species of the group represented by *U. globulariaefolia* Mart. To this group I refer, in addition, the most nearly related forms, *U. Gomzii* D.C., *U. fontana* St. Hil., *U. fusiformis* Warming, lack of material of which permits scarcely more than speculation; also the species *U. tridentata* Sylven, *U. Lindmanni* Sylven, *U. ternata* Sylven, *U. bicolor* St. Hil., and *U. amethystina* St. Hil. In these species, with the possible exception of the last named, there occurs also a more or less strongly developed ramp below the entrance of the bladder. The yellow-flowered *U. modesta* D.C., displays also this type of structure. *U. Selloi* G. Weber, a species of which the bladders have not yet been described, also belongs here. This small, yellow-flowered plant recalls in many ways *U. Lloydii*. Aside from differences in the form of the flower, differences can also be recognized in that of the bladders. Whether dimorphism of the bladders occurs in *U. Selloi* G. Weber, I cannot at the moment say, since I have seen only one stolon bladder. This was relatively long-stalked with three-cornered antennae and a ramp provided with files of hairs in the usual manner. The bifid hairs of *U. Lloydii* are, however, represented by quadrifid hairs, as is usual in the other above-cited species.

The leaf bladder type occurs also in the group of species represented by *U. colorata* Benj. All its representatives, *U. colorata* Benj., *U. Meyeri* Pilger, *U. spicata* Sylven, *U. micrantha* Benj. and probably also *U. dubia* Benj. are yellow flowered and have narrow, bladder-bearing leaves with two-horned bladders without external rows of trichomes and all provided with bifid internal trichomes. The type of bladder provided with horn-like antennae is in any event of very frequent occurrence among the Brazilian *Utriculariae*, as, for example, in *U. reniformis* St. Hil. and *U. Dusenii* Sylven. *U. Lloydii*, therefore stands in the middle position between the *U. globulariaefolia*-*U. modesta* group on the one hand and the *U. colorata* group on the other.

The diagnosis of the species is as follows:

Utricularia Lloydii Merl sp. nov. *Utricularia* gracilis, annua, stolonibus capillaribus, apice rectis, ampulliferis foliiferisque. *Ampullae stolonum* (circ. 0.9×0.7 mm.) in fronte duabus antennis triangulis, glanduloso-pilosis, circa os pilis glandulosis munitae, sub ore menti instar procursu. *Folia* lineari-lingulata, ampullifera, circ. 5-7 mm. longa: lamina circ. 4×0.3 mm. *Ampullae superiores foliorum* in fronte cornigerae, cornibus (antennis) sub longioribus atque tenuioribus quam ampullae stolonum, sine pilis longis extraneis. *Scapus* gracilis circ. 3.8-5 cm. longus, filiformis, parte superiore 0.3-0.4 mm., parte

inferiore — 0.5 mm. crassus, uni- (vel bi-?) florus, paucis (2–4). *Squamis* minutis, lanceolato-ovatis, basifixis, 0.6–0.7 mm. longis. *Bracteae* ternatae, basifixae, bractea media triangulo-ovata, 0.8–1.2 mm. longa, laterales vix conspicuae breviores, linearisubulatae. *Pendunculus* 2–3 mm. longus. *Sepala* subinaequalia, superius ovatum acutum, integrum, circ. $2.4\text{--}2.6 \times 1.3\text{--}1.8$ mm., inferius minus, ovatum, apice minute emarginatum, $2.2\text{--}2.3 \times 1.3\text{--}1.6$ mm. *Corolla* lutea; labium superius integrum, ovatum, subrotundatum, 2.4–3.4 mm. longum, 1.5–1.8 mm. latum; labium inferius integrum, subundulatum, rotundatum, porrectum, 2.8–3.0 mm. longum, diametro transverso 3.7–4.4 mm. latum; palato non valde elevato, rotundato, margine subpiloso; calcare descendente, recto, leviter sursum curvato, acuto, vel apice vix conspicuei praemorso, lab. inf. longiore, 3.6–4.5 mm. longo. Capsula ovata; semina minutissima, subglobosa, striata.

“Brasilia, ad flumen Rio Branco, Retiro do Serra da Lira, locis arenosis humidisque.”

Type specimen in Herbarium of the New York Botanical Garden. Co-type: Botanical Institute, Munich.

Vegetation of the northwestern coast of Mexico

FORREST SHREVE

(WITH THREE FIGURES)

The portion of the mainland of Mexico lying along the Gulf of California and the Pacific Ocean in the states of Sonora and Sinaloa is of ecological and phytogeographic interest by reason of its displaying the transition from desert to sub-tropical thorn forest. The region is one in which the flora has not been thoroughly studied and in which the vegetation has not been described. In collaboration with the Dudley Herbarium of Stanford University, the Desert Laboratory is now conducting an investigation of the flora and vegetation of the desert region surrounding the head of the Gulf of California, comprising portions of Baja California, California, Arizona and Sonora. The present paper embodies a very brief discussion of a part of this territory and of the region immediately south of it with respect to the relations between desert and thorn forest.

Arid conditions prevail from eastern Washington southward through the Great Basin and Mohave Desert to the mouth of the Colorado River, thence continuing along both sides of the Gulf of California into the tropical zone. The pronouncedly desert conditions found north of Lat. 28° are gradually ameliorated by a heavier and better distributed rainfall in the Southern District of Baja California and on the Mexican mainland south of Sonora. A comparison of the northern and southern extremities of the desert reveals an almost complete difference in flora and dissimilarity in the character of the vegetation. In addition to the gradual changes that are found in going south in the Great Basin there is a marked transition in the vegetation in southern Nevada, where a rapid fall in elevation accompanies the supplanting of *Artemisia tridentata* by *Larrea tridentata* and *Franseria dumosa* as the most abundant plants. The transition from desert to thorn forest in southern Sonora brings within a distance of 200 km. a change in the plant life almost as great as that found in the 2400 km. from the Columbia River basin to the valley of the Rio Sonora.

Extensive intermont plains occupy southwestern Arizona and the western half of Sonora, gradually narrowing to a belt only 40 to 60 km. in width throughout the length of Sinaloa. It is in the coastal fringe of the desert below 500 m. elevation that the most comparable locations are found for observing the changes in vegetation which mark the southern limit of the North American desert on the Pacific slope.

The region adjacent to the lower course of the Colorado River has a rainfall of less than 125 mm. (5 in.) per year, and certain parts of it have the thinnest plant covering to be found in North America. The principal

plants of this region are *Larrea tridentata* and *Franseria dumosa*. In many large valleys they outnumber all other perennial plants 50 to 1. In spite of the marked difference in the vegetative characters of these plants they are closely parallel in the variation of their size and abundance from place to place. They are often the dominants in all situations and all types of soil. It has been estimated in several localities that if the crowns of these shrubs and all of their associates were projected on the ground their collective area would be from 4 to 15% of the total surface. Other perennials occur-

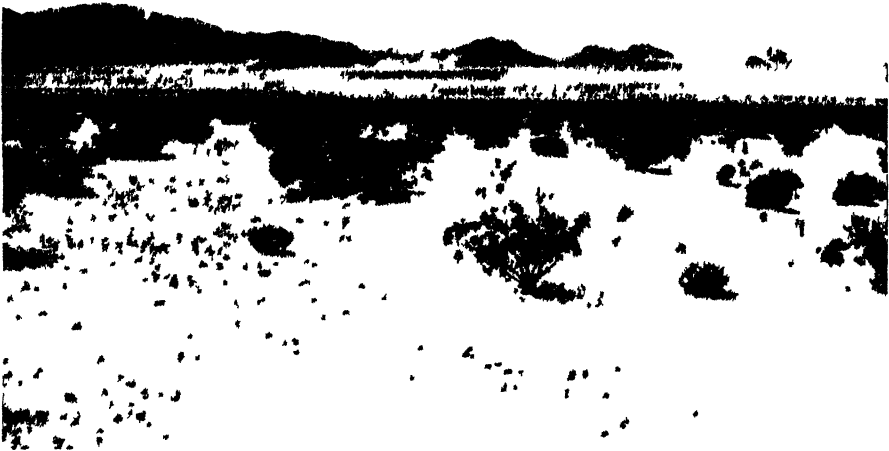


Fig. 1 Typical landscape in the deserts bordering the lower course of the Colorado River, with *Larrea tridentata* and *Franseria dumosa*.

ring in such situations are *Bebbia juncea*, *Petalonyx thurberi*, *Opuntia ramosissima*, *Encelia frutescens* and *Atriplex canescens*. On sandy soil the grass *Hilaria rigida* is abundant, forming large tufts. Where small streamways cross the plains there is a great increase in the number of plants per unit area as well as in the number of species present. Here only are small trees found including *Cercidium torreyanum*, *Olneya tesota*, *Prosopis velutina* and *Parosela spinosa*, as well as the shrubs *Acacia greggii*, *Lycium andersonii* and *Hymenoclea monogyra*.

South of the Gila River the plains of Yuma Co., Arizona, exhibit a somewhat higher type of desert vegetation. The percentage of ground cov-

ered is higher, although the vegetation is still very open, the average height of the perennials is greater, the number of frequent species rises to 15 or 20, the differentiation of the vegetation of the various habitats is higher, and there are representatives of several vegetative types. In these areas *Larrea* and *Franseria* are still present but together form only about 60% of the plant population. Their commonest associates are *Olneya tesota*, *Encelia farinosa*, *Acacia greggii*, *Cercidium torreyanum*, *Hilaria rigida*, *Opuntia ramosissima*, *O. bigelovii*, *Echinocereus engelmannii* and *Krameria grayi*. A complete list of the large perennials of these plains would include about 30 species.

The two similar types of vegetation which have been described are found, with minor variations, throughout the plains of southwestern Arizona and northwestern Sonora as far south as Lat. 30°. In the lower drainage of the Magdalena (Concepcion) River, *Larrea* occurs in extensive stands with no *Franseria dumosa* and few other plants, or in other localities with *Franseria deltoidea*. A few new perennials are added to the flora between the Gila and Magdalena Rivers but none of them are important components of the vegetation of the plains. Among these are *Elaphrium microphyllum*, *Jatropha spathulata*, *Sapium biloculare*, *Cordia greggii*, *Lophocereus schottii*, *Colubrina glabra* and *Lycium richii*.

In the region between the Magdalena and Sonora Rivers the vegetation remains very open but increases slightly in its average height and considerably in the number of abundant species. *Larrea* becomes more closely confined to the immediate coast and to certain restricted habitats, while *Franseria dumosa* reaches its southern limit. The species which are here dominant throughout the plains are chiefly ones that occur north of the Gila River but are there confined to streamways, hills or coarse outwash slopes. The principal change from Lat. 34° to Lat. 30° is therefore the elimination of the highly xeromorphic dominants of the former region and the emergence of the slightly less xeric species from their restricted habitats into the position of dominance. This involves *Olneya tesota*, *Cercidium torreyanum*, *Prosopis velutina*, *Celtis pallida*, *Condalia spathulata*, *Encelia farinosa*, *Lycium andersonii*, *Lemaireocereus thurberi*, *Cercidium microphyllum*, *Acacia greggii*, *Jatropha cardiophylla* and *Elaphrium microphyllum*. Species first appearing in this section of the coast and of importance in the aspect of the vegetation are *Cercidium sonorae*, *Acacia occidentalis*, *Fouquieria macdougalii*, *Pachycereus pringlei*, *Opuntia thurberi*, *Jatropha cinerea* and *Guaiacum coulteri*. The general aspect of the vegetation is determined by the leguminous trees *Olneya*, *Prosopis* and *Cercidium*, and by the shrub *Encelia*. As *Larrea* disappears its place as the most abundant and ubiquitous plant is taken by *Encelia*. Cacti are frequent enough to

lend character to the landscape but form a very small percentage of the plant population. *Opuntia fulgida* is locally abundant but often absent over areas of hundreds of hectares. *Opuntia thurberi* occurs frequently but is nowhere abundant. *Lophocereus schottii* and *Lemaireocereus thurberi* are of frequent occurrence but not continuously within range of the eye. *Rathbunia alamosensis* is abundant only in thickets along the streamways.

From the Sonora River southward nearly to the Yaqui River there is little change in the physiognomy and composition of the vegetation just described. There are still all of the characteristics of the less pronounced



Fig. 2 Open type of arboreal desert near Moreno in central Sonora, with *Prosopis velutina*, *Ichthyomethia mollis* and *Euclea farinosa*

type of desert. The plants are widely spaced, the height of the tallest trees is from 4 to 5 m, trees and shrubs are of strongly xeromorphic character, succulents are frequent, the vegetation is denser along the streamways than elsewhere, annuals and seedling perennials are abundant chiefly in the shade of the largest trees. This belt is noteworthy for the first appearance of *Acacia macracantha*, which is the dominant tree of the thorn forest for hundreds of kilometers to the south, and for the last occurrence of *Larrea*, as a plant of minor importance in the vegetation of alluvial flats near the coast 8 km east of Empalme. The larger streamways of this belt are fringed with trees from 3 to 5 m higher than the general level, or are bordered by thickets. Like the analogous situations far to the north these slightly more favorable habitats induce heavier stands of the plants

common to the region and also support the northernmost examples of many perennials. At about Lat. $28^{\circ}30'$ these situations begin to bear dense thickets of vegetation, with trees, shrubs, herbaceous perennials, cacti and vines, all forming communities such as do not exist further north, and giving a strong forecast of the vegetation found further south. Some of the trees of the riparian thickets are *Acacia macracantha*, *Guazuma ulmifolia*, *Ichthyomethia mollis*, *Pithecolobium sonorae*, *Vitex mollis*, *Coursetia glandulosa* and *Haematoxylon brasiletto*. The arborescent *Pachycereus pecten-aboriginum* here finds its northern limit while *Rathbunia* and *Opuntia thurberi* grow in great profusion, partly supported by the shrubbery. Other woody plants common in the thickets of this belt are *Lantana camara*, *Euphorbia plicata*, *Karwinskia humboldtiana*, *Cordia greggii*, *Mimosa grahami*, *Cassia biflora*, *Paullinia sonorensis* and *Vallesia glabra*.

Between Lat. 28° , in the vicinity of Guaymas, and Lat. 27° , in the vicinity of Navojoa, comes the rapid change which marks the southern limit of the desert. The riparian thickets become more extensive, and the open patches suggestive of desert spacing become fewer. In certain situations, however, they may still be seen as far south as the valley of Rio Fuerte, in Sinaloa, with *Cercidium*, *Encelia* and *Opuntia fulgida* as their characteristic plants. The increase in density involves both trees and shrubs. The maximum height of the vegetation increases slightly. Where there is relative simplicity in the composition, due to the prevalence of *Acacia macracantha*, there is a uniform canopy. More often, however, the sky line is very irregular, due to the increasing complexity of composition and to the difference in the height attained by the secondary trees. The vegetation is nowhere dense enough to bring about the elimination of all but the tallest trees.

The thorn forest is at best a xeric type of vegetation. Its trees rarely exceed 10 m. in height, while the average for the dominants is 7 to 8 m. The crown is rarely dense enough to eliminate small shrubs and root perennials. The trees branch widely and have sparse foliage. The leaves of the common trees are small or more often compound with small leaflets. Cacti are more abundant in the thorn forest than they are in most of the coastal territory between the Magdalena and Yaqui Rivers. Spines and thorns are very prevalent. In spite of these xeric features the thorn forest is rather sharply differentiated from the desert, as it has been characterised in a previous paragraph. Not only does the vegetation serve as a criterion of the difference but also the nature of the weathering, the condition of the soil surface, the accumulation of litter, the evidences of runoff, the character of both the smaller and larger drainageways, and the physiographic influences of wind.

In the thorn forest of the southernmost part of Sonora *Acacia macracantha* is the commonest tree, forming approximately 60 per cent of the stand. It has several important associates which are sporadic in occurrence and uneven in abundance, although there are no easily recognised variations of soil or other features to which their presence can be attributed. Prominent among the secondary trees and other large plants are *Ichthyomeihia mollis*, *Cercidium sonorae*, *Ipomoea arborea*, *Zizyphus sonorensis*, *Pachycereus pecten-aboriginum*, *Prosopis glutinosa*, *Ceiba acuminata*, *Cassia biflora* and *Coutarea pterosperma*.



Fig. 3 Typical thorn-forest 46 miles east of Cajeme in southern Sonora, with *Acacia macracantha*, *Pachycereus pecten-aboriginum* and *Franseria cordifolia*.

For 500 km south of Navojoa, to the southern boundary of Sinaloa, the coastal region is narrow, consisting mainly of mature outwash slopes from the adjacent mountains, crossed frequently by alluvial belts bordering the large rivers. Throughout this distance the physiognomy of the vegetation is almost identical. There is a slight increase in the density, particularly that of the shrubs and other undergrowth, south of the Rio Elota, but the height, character of canopy, nature of the foliage and other general features undergo little change. Many of the common plants of central Sonora do not extend to the southern end of Sinaloa, as *Cercidium torreyanum*, *C. sonorae*, *Olneya tesota*, *Fouquieria macdougalii*, *Encelia farinosa*, *Lemaireocereus thurberi*, *Forchammeria watsoni*, *Lophocereus schottii* and *Pithecolobium sonorae*. A dominant place is held by *Acacia macra-*

cantha, *Ipomoea arborea* and *Zizyphus sonorensis* throughout Sinaloa, and several new secondary trees appear, notably *Lonchocarpus megalanthus*, *Bauhinia longiflora*, *Crescentia alata*, *Bunchosia palmeri* and *Caesalpinia cacalaco*. The commonest of the shrubs is *Croton alamosanus*. Striking features of the vegetation in southern Sinaloa are the frequency of native palms, several epiphytic species of *Tillandsia*, and also numerous terrestrial bromeliads. The alluvial plains bear scattered examples of trees of much greater height and size than those of the thorn forest.

In considering the physical conditions which control the vegetation through the 1500 km. from the Bill Williams valley in western Arizona to the valley of the Rio Union in southern Sinaloa it is obvious that both rainfall and temperature are of great importance. There is a gradual increase in the annual precipitation from a range of 50 to 125 mm. (2 to 5 in.) north of the Magdalena River, and 250 to 375 mm. (10 to 15 in.) between there and the Yaqui River, with a marked increase in southern Sinaloa to 722 mm. (28.42 in.) at Mazatlan. The percentage of the annual total which falls in the summer months increases from 25 in the north and 75 in southern Sonora to 77 in southern Sinaloa. Much of the importance of the increasing rainfall for plants is offset by the rising temperature and increasing length of the hot period which accompanies it. The transition from desert to thorn forest coincides very nearly with the attainment of an annual rainfall of 375 mm. (15 in.). This is a datum, however, which is of little importance except in connection with accompanying conditions.

With reference to temperature it must be noted that the entire area under discussion is subject to high daytime ranges during six to nine months of the year, according to latitude. Frost is of annual occurrence north of the Magdalena, but becomes less frequent through southern Sonora. It is infrequent at Cajeme (Ciudad Obregon), Son., and rare at Los Mochis, Sin., being unknown further south.

The short distance within which desert gives way to thorn forest, as contrasted with the extended range of each of these vegetations to the north and south, seems to indicate the operation of a potent group of controlling conditions. The rise of rainfall, particularly in the summer months, the change from a continental to a coastal position (due to the location of the southern end of the peninsula of Baja California), the decreasing distance from tide water to a high mountain background, and the absence of frost are undoubtedly all concerned. The plants of southwestern Arizona are both drought resistant and cold resistant. Those of Sinaloa are drought resistant only. It appears highly probable that the winter temperature conditions have strongly limited the number of drought re-

sistant plants that have been able to enter the desert, and that the relatively small flora of the desert is not to be attributed solely to adverse moisture conditions.

SUMMARY

The most arid region in North America lies along the lower course of the Colorado River and on both sides of the head of the Gulf of California. In portions of this region *Larrea tridentata* and *Franseria dumosa* form 98% of the vegetation but often cover only 4 to 15% of the ground surface. The less frequent plants are confined to streamways, coarse outwash and mountain slopes, but almost all of them are found in the vegetation of the plains in Sonora south of Lat. 30°. There the dominant plants are *Olneya tesota*, *Cercidium torreyanum*, *Prosopis velutina* and *Encelia farinosa*.

On going south from central Sonora there is a steady increase in the number of common perennials and in the number of vegetative types that are represented. Cacti are locally abundant but often absent over large areas or found only as occasional large individuals.

At about Lat. 28°30' the vegetation of streamways and alluvial plains begins to be much more dense and many new trees and shrubs are found in such situations, the majority of them being forms that are dominant in the general vegetation further south. Between Lat. 28° and Lat. 27° there is a rapid change in the character of the vegetation, which here becomes denser, taller and richer in composition, at the same time that a few less xeromorphic species appear. This is the region of transition from desert to the arid type of thorn-forest which extends southward from the Mayo River to southern Sinaloa, with little change in its physiognomy and ecological characteristics but with constant additions to its flora.

It is within a relatively short distance that the extended area of desert merges into thorn-forest, a type of vegetation also of wide distribution on the Pacific coast of Mexico. The transition coincides closely with the attainment of an annual rainfall of 375 mm. and the disappearance of frost.

THE DESERT LABORATORY
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Haploid chromosomes of *Riccia glauca*

D. A. JOHANSEN

(WITH TEXT-FIGURE)

Riccia glauca L. is increasingly coming into favor among instructors as being more satisfactory than *Ricciocarpus natans* for the demonstration of the origin and development of the sex organs and spores in a simple liverwort. Some instructors, requiring the observation of greater detail than do others, have met with difficulty when attempting to demonstrate the reduction of the chromosome number during spore formation. The trouble is that the haploid number of chromosomes, as judged from the scanty literature available, appears to be in dispute. The present paper constitutes an attempt to provide an answer to the question as to exactly how many haploid chromosomes *Riccia glauca* possesses.

Beer (1906) states that "The reduced number of chromosomes . . . is either seven or eight, but it could not be decided with certainty between these two numbers." Heitz (1927), using his peculiar boiling method, claims to have found nine haploid chromosomes, while Miss Wentzel (1929) cites the number as being eight.

During the past few years the writer has prepared many hundreds of slides of *R. glauca* for class purposes. While checking over and classifying these preparations, all slides showing the reduction divisions in the spore mother cells were set aside for future study. In this manner a collection of about 60 slides, representing approximately the same number of individual plants, has accumulated and forms the basis of the following report.

The plants were collected at various localities in west-central California, and at one locality in Southern California. Except for the larger size of the southern plants, they were much alike and showed no apparent cytological differences.

Fixation in some cases was with a formalin—acetic acid—50% alcohol mixture; in others, the plants were allowed to remain in Carnoy's fluid for not more than six minutes and were then transferred to Navashin's fluid. The sections were cut at 12 microns. Some slides were stained with iron haematoxylin, others with methyl violet and erythrosin by the picro-alcohol method.

In all cells showing the early first metaphase in polar view (fig. 1), eight chromosomes are to be found. The chromosomes may be divided into three size groups, namely, two large chromosomes, five of a slightly smaller size and a single tiny globular element. The tiny chromosome divides precociously at mid-metaphase (fig. 2). During the anaphase (fig. 3), the two

tiny chromosomes, which go to opposite poles, invariably precede all the other chromosomes. The two larger chromosomes lag behind the five in the next size group, and one of the two has frequently been observed to delay final separation until all the other chromosomes have already reached their respective poles and the interphase is about to occur.

The writer feels convinced that the true haploid number of chromosomes in *Riccia glauca* is eight. Beer (1906) plainly had difficulty in recognizing the tiny eighth chromosome. As for the count of nine claimed by Heitz (1927), there are two possible explanations. The less probable one

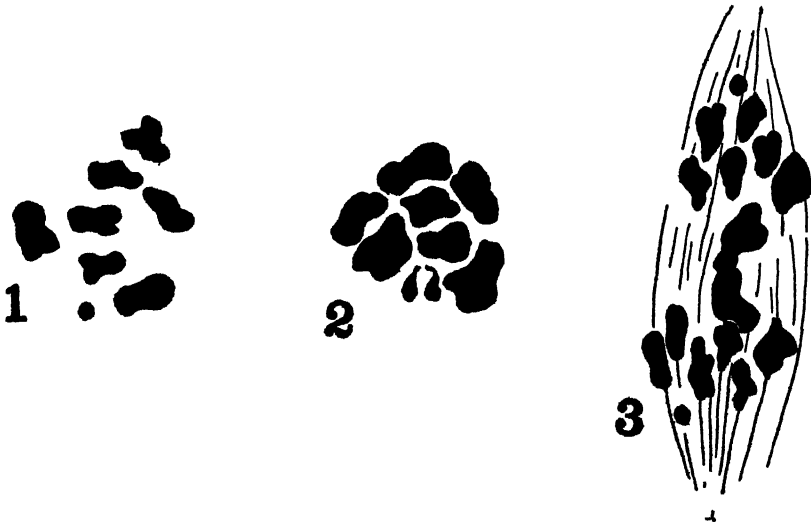


Fig. 1. Early metaphase I in spore mother cell of *Riccia glauca*. Fig. 2. Late metaphase I showing division of tiny eighth chromosome. Fig. 3. Anaphase I showing delayed separation in one of the two large bivalents. $\times 4400$.

is that his observations were made at late metaphase after the tiny globular element had divided. It appears more likely however, to judge by the badly shrunken appearance of the chromosomes in his fig. 61, that fixation was so incredibly poor that a misinterpretation was made. Only one large chromosome is shown, whereas in the writer's preparations two large elements were always observed. It is probable that the two vertically oriented chromosomes at the left in the figure cited are in reality a single chromosome, as their combined length about equals that of the one large chromosome. Miss Wentzel (1929) claims that her "Figur 8 zeigte die Polansicht einer Anaphase mit 8 Chromosomen." The figures are so badly reproduced as to be useless; in this particular figure, the stage appears to be the metaphase but the chromosomes are indistinguishable from the dots used for

stippling. The same criticism applies to her figures 7 and 9, in which "In die seitlichen Ansichten der Spindeln sind bei einer Einstellung des Mikroskops 3-5 Chromosomenpaare zu sehen."

The writer is indebted to Mr. Louis C. Wheeler for the material from Southern California, and to Dr. Peter Claussen of Marburg, Germany, for invaluable assistance with certain literature which would otherwise have been unavailable.

Literature cited

- Beer, R. 1906. On the development of *Riccia glauca*. Ann. Bot. 20: 277-291.
Heitz, E. 1927. Ueber multiple und aberrante Chromosomenzahlen. Abhandl. Naturw. Ver. Hamburg 21: 47-58.
Wentzel, R. 1929. Die Tetradenbildung bei *Riccia glauca* L. und bei *Antihoceros levis* L. und *punctatus* L. mit besonderer Berücksichtigung der Membran-entstehung. Diss., Marburg-Lahn. 60 p.

A revision of the subgenus *Hugelia* of the genus *Gilia* (Polemoniaceae)

THOMAS CRAIG

(WITH PLATE 23)

This study, carried on at the Pomona College Herbarium, was undertaken at the suggestion of Dr. P. A. Munz. I am very much indebted to him for guidance and assistance throughout this undertaking, and I am also indebted to the following persons for supplying notes and for lending herbarium specimens: Dr. H. L. Mason and Elizabeth Crow Norland of the University of California (C.), Miss Alice Eastwood of the California Academy of Sciences (C.A.), Dr. Aven Nelson of the University of Wyoming (Wyo.), Dr. Wm. R. Maxon of the United States National Herbarium (U.S.), Mr. Frank Peirson of Pasadena (F.P.), and Dr. C. B. Wolf of Rancho Santa Ana (S.A.). The abbreviations in parentheses are those used in the citation of specimens. I have also visited the herbaria of Stanford University (S.), and the Santa Barbara Museum (S.B.), in order to study the collections of *Hugelia* in these herbaria. In addition to the work carried on in the herbarium, much time and effort have been spent in the field; I have seen a large percentage of the forms growing.

HISTORY OF THE SUBGENUS

The history of the subgenus *Hugelia* has been reviewed by Macbride, Cont. Gray Herb. n.s. 49: 54. 1917. Therefore, for the sake of brevity, I am giving only the following short synopsis to cover the history of the group.

HUGELIA

Hugelia, as a new genus, was proposed by Bentham, Bot. Reg. 19: sub. t. 1622. 1833, in honor of Baron Charles de Hügel of Vienna. As section, Gray Proc. Am. Acad. 8: 272. 1870. As subgenus under *Gilia*, Milliken, Univ. Calif. Pub. Bot. 2: 24, 38-40. 1904. As subgenus under *Navarretia*, Brand, Pflanzenreich IV, 250: 164-168. 1907. *Eriastrum*, Wootton & Standley, Contrib. U. S. Nat. Herb. 16: 160. 1913. *Welwitschia*, Rydb., Fl. Rocky Mts. 688, 1065. 1917. *Hugelia* as section under *Gilia*, Macbride, Cont. Gray Herb. n.s. 49: 54. 1917. As genus, Jepson Man. Fl. Pls. Calif. 792-793. 1925.

I cannot agree with Brand in his inclusion of *Hugelia* in *Navarretia*, while at the same time separating both from *Gilia*, even though the diffusely branched *G. filifolia* var. *typica* and *Navarretia Abramsii* are so closely related as to appear almost conspecific; for other *Hugelias* are also

closely related to other groups of *Gilias*. *Gilia filifolia* var. *diffusa*, especially in plants from Utah and Texas, very closely approximates *G. Gunnisonii*. *Gilia densifolia* is rather close to *G. Wrightii*, and *G. eremica* is much like a *Langloisia*. Because these groups are all so closely related, and because of the lack of absolute characters to separate the groups, I believe it better to regard *Hugelina* not as a part of the genus *Navarretia*, as done by Brand; nor as a separate genus, as done by Jepson, l.c., but as a subgenus of *Gilia*.

KEY TO SPECIES

- A. Woody-based perennial; corolla 15–30 mm. long; the tube 2–3 times longer than calyx.

1. *G. densifolia*.

- AA. Annual; corolla 7–18 mm. long; the tube rarely longer than calyx, never more than twice as long.

- B. Flowers yellowish; the corolla lobes almost equalling the tube; anthers more than $1\frac{1}{2}$ mm. long. Central & So. Calif. cismontane region and high desert slopes of So. Mts.

3. *G. lutescens*.

- BB. Flowers not yellow, or if yellowish, as is rarely the case with forms from desert regions, the corolla lobes are half the length of the tube, and the anthers 1 mm. or less long.

- C. Free portions of shortest stamen at least as long as corolla tube; corolla lobes as long as tube, blue or pale lavender. Cismontane and high desert regions of So. California.

2. *G. virgata*.

- CC. Free portions of the shortest stamen not more than two thirds the length of the corolla tube.

- D. Corolla 6–8 $\frac{1}{2}$ mm. long, commonly 7 mm. Anthers $\frac{1}{2}$ –1 mm. long.

- E. Stems erect from base.

- F. Lower cauline leaves 3–5-lobed, the lobes commonly at least $\frac{1}{2}$ mm. broad.

8. *G. Wilcoxii*.

- FF. Lower cauline leaves entire or 3-lobed, the lobes less than $\frac{1}{4}$ mm. broad.

7. *G. filifolia*.

- EE. Stems decumbent from base.

7. *G. filifolia*.

DD. Corolla 10 mm. long or longer, commonly 12–17 mm. Anthers rarely less than 1 mm. long, commonly about 2 mm.

E. Flowers quite irregular, the free portion of shortest stamen less than half as long as the free portion of longest stamen.

6. *G. eremica*.

EE. Flowers regular or nearly so, stamens of almost equal length.

F. Lower cauline leaves 3–7-parted, most commonly 5-parted.

G. Flowers deep blue violet, lobes rarely less than two thirds as long as tube; leaf lobes commonly slender filiform, commonly up to 10 mm. long. Central Calif. valleys.

4. *G. pluriflora*.

GG. Flowers pale vivid blue or lavender, the lobes not more than $\frac{1}{2}$ length of tube. Leaf lobes rarely slender filiform and rarely more than 8 mm. long.

H. Corolla 10–13 mm. long, commonly 11 mm. Anthers 1 mm. or less in length. N. E. Calif., Nev., Utah, and Northward to Wash. and Idaho.

8. *G. Wilcoxii*.

HH. Corolla $14\frac{1}{2}$ –17 mm. long. Anthers 1 mm. or more in length. South-western Mojave Desert.

5. *G. Sherman-Hoytae*.

FF. Lower cauline leaves commonly entire or with two basal lobes, very rarely with an occasional leaf with 4 basal lobes.

G. Stamens of slightly unequal length, corolla pale blue or lavender. Arizona.

6. *G. eremica*.

GG. Stamens sub-equal, corolla deep blue. Calif.

2. *G. virgata*.

TREATMENT OF SPECIES

1. *GILIA DENSIFOLIA* Benth., in D. C., Prodr. 9: 311. 1845. *Hugelia densifolia* Benth., Bot. Reg. 19: sub. t. 1622. 1833; *Navarretia densifolia* (Benth.) Brand, Pflanzenreich IV, 250: 165. 1907; *Gilia Hugelia* Steud., Nom. ed. 2, 1: 683. 1840.

Erect perennial herb, 8–90 cm. high, much branched from woody base, glabrous to canescent lanate; stems rigid; leaves 1–6 cm. long, lanceolate to almost ovate in outline, pinnatifid into subulate spinulose or pungent lobes, rarely narrowly linear and entire; inflorescence capitate-glomerate, pilose to densely lanate; calyx from $\frac{1}{3}$ the length of the tube to almost equalling it; corolla salverform, 15–30 mm. long, the oblong lobes $\frac{1}{2}$ – $\frac{2}{3}$ the length of the tube, deep violet blue to pale lavender; stamens inserted just below the sinuses, not exceeding lobes; anthers sagittate, 1.5–2.5 mm. long; filaments 4–5 mm. long; capsule 3–4 mm. long.

The perennial habit of this species distinguishes it from all other species of *Hugelia*. The only possible sources of confusion are occasional specimens of *G. virgata* var. *dasyantha* Brand., which plants, due to fall and winter rains, will on rare occasions survive and bloom in late winter and thus remotely resemble a perennial. The species is common in Central, Southern, and Lower California.

KEY TO VARIETIES

- A. Corolla 22–32 mm. long. Plants from sea level—1500 ft.
 - B. Corolla 25–32 mm. long, lobes one third as long as tube, plants lanate; from Santa Ana River Wash. in So. Calif. at elevations of 500–1500 ft.
 - 1b. *G. densifolia* var. *sanctorum*.
 - BB. Corolla 22–25 mm. long, lobes almost $\frac{1}{2}$ as long as tube, plants glabrous or nearly so. Coastal San Luis Obispo & Santa Barbara Counties.
 - 1c. *G. densifolia* var. *typica*.
- AA. Corolla 14–19 mm. long. Plants from sea level—8000 ft.
 - B. Leaves commonly recurved, pinnatifid; the lobes about as long as width of rachis; fls. pale blue or lavender. Plant canescent lanate. Mohave Desert.
 - 1e. *G. densifolia* var. *mohavensis*.
 - BB. Leaves usually ascending, lobed or entire; the lobes 2–10 times as long as width of rachis; fls. deep blue. Plant lanate to sub-glabrous.
 - C. Bracts of fl. clusters sometimes entire, commonly 3–5-lobed, very rarely 7-lobed; plants 20–90 cm. high; many axillary clusters, which are rarely more than 10-flowered, never more

than 15-flowered, leaves lobed or entire. Plant always remaining thinly canescent-lanate in age. Coastal and interior valleys below 4000 ft.

1a. *G. densifolia* var. *elongata*.

CC. Bracts of fl. clusters commonly 5–9-lobed; plants 15–45 cm. high; flower clusters mainly terminal, commonly 15-flowered sometimes as much as 20-flowered; leaves 2–15-lobed, never entire. Plant not uncommonly sub-glabrous. So. Calif. Mts. from 4000–8000 ft.

1d. *G. densifolia* var. *austromontana*.

1a. *GILIA DENSIFOLIA* Benth., var. *ELONGATA* (Benth.) Gray ex Brand, *Pflanzenreich* IV, 250: 165. 1907; *Hugelia elongata* Benth., Bot. Reg., l.c. *Gilia elongata* (Benth.), Steud, Nom. ed. 2, 1: 683. 1840; *Navarretia densifolia* subsp. *elongata* Brand, *Pflanzenreich* IV, 250: 165. 1907; *Gilia densifolia* var. *elongata* (Benth.) Gray ex Brand, l.c.

Plants 20–90 cm. high, canescent-lanate when young, remaining so at least in the upper half until maturity; leaves ascending, rarely narrowly linear or, more commonly, with linear spinulose lobes more than twice the width of the rachis; flower clusters comparatively small rarely as much as 15-flowered, lateral as well as terminal; corolla deep blue to deep blue-violet, 15–16 cm. long, very rarely 20 mm. long; lobes half the length of the tube; stamens $4\frac{1}{2}$ –5 mm. long.

Type locality, California, the type collection having been by Douglas, hence from coastal region between Santa Barbara and Monterey. Ranging at lower elevations along the coast from San Luis Obispo Co. to Lower Calif. and into the interior as far as San Bernardino Valley and Southern Mohave Desert. Representative material studied: CALIFORNIA: Monterey Co., Tassajara, *Dudley* in 1901 (S); Paloma Creek, Santa Lucia Mts., *Bacigalupi* (S). San Luis Obispo Co., Templeton, *Abrams* 5044 (P); Creston, *Feudge* 1743 (P). San Benito Co., Hernandez, *Lathrop* in 1902, and 1903 (S); Cooks 7 mi. from Pinnacles, *Abrams* 6702 (S). Kern Co., Between Bakersfield and Bodfish, *Abrams* 5349 (S). Santa Barbara Co., Near Lompoc, *Mc Minn* 1047 (S); Santa Inez River, San Marcos Ranch, *Hoffmann* (S.B.). Los Angeles Co., Roscoe, *McFadden* in 1931 (C); Saugus, *Munz* and *Johnston* 11,132 (P); Pacoima Wash, San Fernando, *Munz* 9387 (P); Junction Soledad & Mint Canyon Roads, *Craig* 462 (P); Mint Canyon, *Craig* and *Hilend* 525 (P); Aliso Canyon, *Barber* 202 (C), Soledad Canyon 8 mi. W. of Ravenna, *Craig* 2007 (P). San Bernardino Co., West Slope Cajon Pass, $\frac{1}{4}$ mi. from Summit, *Craig* 1470 (P); 1 mi. from Summit, *Craig* 1471 (P); Cajon Pass, 1 mi. above Camp Cajon, *Craig* 1472 (P);

Cajon Pass $\frac{1}{2}$ mi. below Camp Cajon, *Craig* 1473 (P); Cajon Pass 1 mi. above Devore, *Craig* 1474 (P); Cajon Pass Wash at Highland Blvd., *Craig* 1475 (P).

The last two numbers cited are intermediate with *sanctorum* as are all the following numbers from this County. Colton, *Wilder* in 1904 (P), *Hall* 157 (C); Banning, *Jones* in 1903 (P); San Bernardino, *Abrams* 1963 (P), *Parish* in 1888 (C); Etiwanda, *Jones* in 1925 (P). LOWER CALIFORNIA: Tecate, *Orcutt* in 1885 (C), *Orcutt* in 1883 (C). (Shows a tendency toward *austromontana*.)

The following specimens are intermediate between *elongata* and *austromontana*: Los Angeles Co., Acton, Mt. Gleason, *Elmer* 3692 (P); Ravenna, *Brandegee*, June to Aug. LOWER CALIFORNIA: Mts. of Northern Lower Calif., *Orcutt* (C); San Pedro Martir Mts., *Robertson* 49 (C). CALIFORNIA: San Diego Co., Jacumba, *Cleveland* in 1884; Eastern base San Jacinto Mt., *Hall* 1861 (C); Old Nicolas Canyon, *Munz* 5924 (P, C). Riverside Co., between Vandeventer and Hemet Valley, *Munz* 5820 (C, P); El Toro Mt., *Van Deventer*, in 1901 (C). NEVADA: Nye Co., Pahute Peak, *Purpus* in 1897.

1b. *GILIA DENSIFOLIA* VAR. *SANCTORUM* Milliken, Univ. Calif. Pub. Bot. 2: 39. 1904. *Hugelia densifolia* var. *sanctora* (Mill.) Jeps., Man. Fl. Pls. Calif., 792. 1925.

Plant 25–75 cm. high, woody at base, entire plant, except old woody portions, lanate even in age, densely so when young; flowers blue, 25–32 mm. long; corolla-lobes one third as long as the tube.

Type locality, Spanish town crossing above Riverside. The variety in its best development is found in the river bottoms of the Santa Ana River and its tributaries from near Highland, San Bernardino Co. (El. about 1500 ft.), to Rancho Santa Ana, in Orange County (El. 500 ft.). Representative material studied, CALIFORNIA: San Bernardino Co., Santa Ana River near East Highland, *Feudge* 1569 (P); Plains near San Bernardino, *Lemmon* in 1876 (C); San Bernardino, *Parish* 4178 (C); Lytle Creek near San Bernardino, *Parish* 7139 (P, C). Riverside Co., Santa Ana River bottoms, *Reed* in 1910 (P); Santa Ana River near Spanish town crossing, *Hall*, 173 & 683, type (C). Orange Co., Rancho Santa Ana, *J. T. Howell* 2985 (P, S.A.).

1c. *Gilia densifolia* var. *typica* n. nom. *Hugelia densifolia* Benth., Bot. Reg. 19: sub. 1622. 1833. *Gilia densifolia* Benth., in D. C., Prodr. 9: 311. 1845.

Plant 20–30 cm. high, glabrous to sub-glabrous; leaves entire or divided; flower clusters large, mainly terminal, often 25-or-more-flowered; corolla 22–25 mm. long; the lobes about 8 mm. long.

Type locality, California, the type collection having been made by Douglas, hence from the coastal region between Santa Barbara and Monterey. A photograph of this type and one of *elongata* have been obtained from Kew. Representative material studied, CALIFORNIA: San Luis Obispo Co., Haynes Ranch, *Ingalls* in 1912 (C.A.); Sand Hills 2 mi. south of Pismo Beach, *Peirson* 2224 (F. P. P.); Morro Sands, *Eastwood* 14,944 (C.A.). Santa Barbara Co., Beach near Santa Maria, *Eastwood* 867 (C.A.); 3 mi. north of Guadalupe, *Craig* 1926 (P).

The following collections are intermediate between var. *elongata* and var. *typica*: Santa Barbara Co., Lompoc, *Hoffmann* (S.B.); Mesa 3 mi. east of Lompoc, *Breneiser* in 1933 (P); 14 mi. E. of Santa Maria, *Craig* 1873 (P).

This variety resembles var. *sanctorum* in the largeness of its showy flowers. It is however amply distinct in being glabrous or sub-glabrous and in having the corolla lobes longer in relation to the tube, as well as in its more northern range.

1d. *Gilia densifolia* Benth. var. *austromontana* Craig n. var. *Navarretia densifolia* subsp. *eu-densifolia* Brand., Pflanzenreich IV, 250:165. 1907.

Plants 10–45 cm. high, glabrate or but slightly lanate, especially around the heads; leaves ascending, 15–30 mm. long, the spinose lobes from 2–15, usually 6, twice to many times longer than the width of the rachis; flower clusters almost entirely terminal, frequently as much as 20-flowered; corolla deep blue, 15–19 mm. long; lobes 5–6 mm. long; stamens 3–4 mm. long. (Plantae 10–45 cm. altae, glabratae; capitulis leviter lanosis, terminalibus; foliis ascendentibus, 15–30 mm. longis; lobis spinosis, 2–15, saepissime 6, linearibus aut anguste lanceolatis, 3–12 mm. longis; lobis 5–6 mm. longis; staminibus 3–4 mm. longis.)

Type, from dry slope near Nellie, Palomar Mts., San Diego Co., Calif., at 5000 ft. alt., *P. A. Munz* 8341, in 1924, Pomona College Herbarium No. 48,414. Ranging most commonly in the mountains, at altitudes of from 4000–8000 ft., from Los Angeles and San Bernardino Counties to Northern Lower California, being most representative in the southern part of its range. Representative material studied, LOWER CALIFORNIA: Tanqui Canyon, *Jones* in 1928 (P). CALIFORNIA: San Diego Co., Laguna, *Cleveland* in 1885 (C); Palomar Mts., *Spencer* (P); Palomar Mt., *Chandler* 5372 (C), *Jones* in 1926 (P). Riverside Co., Tahquitz ridge, San Jacinto Mts., *Spencer* in 1923 (P); Idyllwild, San Jacinto Mts., *Jones* in 1924 (P), *Hall* 2384 (P); Strawberry Valley, San Jacinto Mts., *Grant* in 1901 (C); Hemet to Mt. San Jacinto, *J. T. Howell*, 574 (S.A.); 6 mi. southeast of Poppet Flat, *Munz* and *Johnston* 8837 (P). San Bernardino Co., Barton Flats, San Bernardino Mts., *Munz* and *Johnston* 9658 (P); Clark's, San

Bernardino Mts., *Jones* in 1900 (P); Green Valley, San Bernardino Mts., *Abrams* 2061 (P); Cactus Flats, San Bernardino Mts., *J. T. Howell* 334 (S.A.), *Johnston* in 1924 (P); Foresee Creek, San Bernardino Mts., *Grinnell* 42 (C); Seven Oaks, San Bernardino Mts., *Parish* 3685 (C), *Hall* 956 (P); Trail East of Hook's Hill, San Bernardino Mts., *Wilder* 317 (P); Coldwater, fork Lytle Creek, San Antonio Mts., *Johnston* 1385 (C, P), Coldwater Canyon, San Antonio Mts., 2682 (P, C); San Antonio Canyon; *Johnston* 1587 (C, P); Head of South Fork of Lytle Creek, San Antonio Mts., *Johnston* 1459 (P).

The following collections are atypical approaching var. *elongata*. CALIFORNIA: Los Angeles Co., Mt. Wilson, San Gabriel Mts., *Grinnell* in 1917 (P). Ventura Co., Cuddy Valley, Mt. Pinos, *Epling* and *Dunn* in 1931 (P); Upper Lockwood Valley, Mt. Pinos region, *Dudley* and *Lamb*, 4677 (S). Santa Barbara Co., Casmalia, *Eastwood* 867, and 758 (C.A.). Monterey Co., Santa Lucia Mts., *Eastwood* in 1893 (C); Santa Lucia Mts., Chew's Ridge, *Rowntree* in 1929 (P).

Although this variety intergrades freely in the lower altitudes with var. *elongata*, a long series shows it distinct enough to warrant recognition. It differs from var. *elongata* in the following ways: it is less woolly, lower growing, less branched above; the heads are larger and there are fewer axillary heads; the leaves have on the average 2-4 more lobes.

1e. *Gilia densifolia* Benth. var. *mohavensis* Craig n. var.

Plants low (8-30 cm.) canescent-lanate, stems brittle, densely much branched, ascending; leaves ovate-lanceolate in outline, 1-5 cm. long, reflexed and rigid, with the 2-8 short spinose lobes rarely longer than the width of the rachis; heads numerous, from 25 in very small plants up to 200 in large ones; corolla pale blue or pale lavender sometimes almost white, 15 mm. long, the lobes less than half as wide as long and about half the length of the tube; stamens $4\frac{1}{2}$ -5 mm. long. (Humilis, 8-30 cm. alta, canescenti-lanosa; caulibus facile fractis, dense et multe ramosis, ascendentibus; foliis ovato-lanceolatis, 1-3 cm. longis, reflexis et rigidibus; lobis foliorum 2-8, brevibus, spinosis, 1-4 mm. longis; rhachidibus 2-3 mm. latis; capitulis numerosis, 25-200; corollis pallide coeruleis, 15 mm. longis; lobis corollarum 5 mm. longis, 2 mm. latis; staminibus $4\frac{1}{2}$ -5 mm. longis.)

Type, from sand dunes between Rosamond and Mohave, Kern Co., Calif., *T. Craig* 1360, June 7, 1928, Pomona College Herbarium No. 182, 123; isotypes, University of California 494,768, and Pomona 182,264. Ranging rather widely over the Mohave Desert and most abundant in Antelope Valley. Representative material studied, CALIFORNIA: Inyo Co., Independence, *Peirson* in 1917 (F.P.); Lone Pine, *Jones* in 1897 (P). Kern Co., Red Rock Canyon, *Howell* 3190 (C.A.), *Hart* in 1926 (C.A.);

Sand Hills south of Mohave, *Munz* 11,069 (P); Betwen Rosamond & Mohave, *Newsom* in 1933 (P). Los Angeles Co., Ten miles south of Muroc, *Munz* and *Craig* 12,929 (P); Lancaster, *Hart* in 1925 (C.A.); Palmdale, *Abrams* and *McGregor* 518 (S). San Bernardino Co., Sand Dunes, Granite Mts., So. of Kelso, *Newsom* and *Hilend* in 1930 (P); The Pipes near Morongo Valley, *Jaeger* and *Keck* 244 (P).

The following sheets are quite intermediate with var. *elongata* and are of interest in that they show the relationship of var. *mohavensis* to *G. densifolia* rather than to *G. eremica* which it somewhat resembles in its low stature. Cameron, *Jones* in 1900 (P); Grade above Hackberry Canyon 8 mi. from Caliente, *K. Brandegee* in 1910; Warner's Hot Springs, *Buttle* in 1913 (C.A.); Edge of San Gabriel Mts., 3 mi. So. of Vincent, *Craig* 2029 (P); Vincent, *Craig* 2031 (P).

Specimens of var. *mohavensis* from the Antelope Valley and Granite Mts., show extreme variation from any of the other varieties of *G. densifolia*, and single sheets from these areas, but for the perennial habit, could not be recognized as belonging to this species, being much shorter, very compactly branching, and generally much paler in color in stem, leaf and flower. The plant is very brittle. The leaves are different in shape as already noted, and are strongly and rigidly recurved.

2. *GILIA VIRGATA* (Benth.) Steud., nom. ed. 2, 1:684. 1840. *Hugelia virgata* Benth., Bot. Reg. 19: sub. t. 1622. 1833; *Navarretia virgata* (Benth.) Brand, Pflanzenreich IV, 250:167. 1907.

Plant annual, 8–50 cm. high, virgate to much branched; leaves filiform and entire, or parted into 3 filiform lobes; flower clusters 3–20-flowered; flowers pale sapphire blue to deep ultramarine, 8–15 mm. long; the lobes about half the length of the tube or longer; anthers linear sagittate.

G. virgata is the most common of all the annual *Hugelias*. There are a number of geographical races of the species, as would be expected in a species of such a variable group and with such a varied life condition, for *G. virgata* is found in almost all life conditions throughout cismontane Central and Southern Calif. and is represented by one variety on the western Mohave Desert. In one form or another it occurs from sea level to altitudes of over 8000 ft. Of these races, I am recognizing four as distinct enough to warrant varietal recognition. Of course, much intergradation occurs between these varieties; there is also some evidence of intergradation of *virgata* with other species; such as *G. filifolia* var. *typica* with *G. virgata* var. *typica*, and of *G. eremica* with some of the varieties of *G. virgata* from the south eastern part of its range. *G. virgata* blooms from late May through August, of very rarely as late as December.

KEY TO VARIETIES

A. Flower heads 1-3-very rarely 4-flowered. Stamens commonly of slightly irregular length. Corolla usually less than 12 mm. long; the lobes usually as long as tube.

B. Calyx always lanate. Bracts 2-5-lobed, 3-flowered heads common, corolla usually less than 10 mm. long.

2d. *G. virgata* var. *ambigua*.

BB. Calyx rarely at all lanate, sometimes thinly so, frequently glandular, bracts 1-3-lobed, 3-flowered heads rare, corolla 10-12 mm. long.

2c. *G. virgata* var. *sapphirina*.

AA. Flower heads 3-20-flowered. Stamens equal or very nearly equal in length. Corolla usually more than 12 mm. long; the lobes usually shorter than the tube.

B. Stem virgate commonly simple or with few branched (1-6) flower clusters; lobes of the corolla more than one third as wide as long. Coastal Central Calif.

2a. *G. virgata* var. *typica*.

BB. Stem always branching; flower clusters 5-100; corolla lobes less than one third as wide as long. Cismontane So. Calif.

2b. *G. virgata* var. *dasyantha*.

2a. *Gilia virgata* (Benth.) Steud var. *typica* n. nom. *Hugelia virgata* Benth., Bot. Reg. 19: sub. t. 1622. 1833; *Navarretia virgata* (Benth.) Brand, Pflanzenreich IV, 259:165. 1907; *Navarretia densifolia* var. *lanata* Brand. l.c.

Plant white floccose, glabrate with age; stem usually simple and virgate 8-50 cm. high, or rarely branched from base and paniculately branched above; leaves slender, 15-50 mm. long, ascending often appressed to stem, entire, filiform, or more rarely parted into 3 linear lobes; flower clusters commonly terminal, or more rarely axillary, 3-20-flowered, surrounded by tripartate bracts 15-20 mm. long; calyx 8 mm. long; flowers regular blue 15 mm. long; the lobes 6 mm. long, more than one third as wide, stamens 5 mm. long, attached 1-2 mm. below sinuses; anthers 2 mm. long linear-sagittate.

Type locality, California, Douglas; probably near Monterey. Range, *G. virgata* var. *typica* is found in Coastal Monterey Co. Toward the south it merges with var. *dasyantha*. These intergrades can be found as far south as Ventura Co. Representative material studied, CALIFORNIA: Monterey Co., California, Douglas (C); Tularcetes, Dudley in 1901 (S); Carmel Valley, McGregor 93 (S); near Monterey, Brewer, in 1861 (C); Pajaro Hills, Chandler 454 (C); Seaside, Del Monte, Heller in 1903 (P); Seaside, Monterey, Eastwood 164 (C.A.); Carmel River, Dudley in 1905 (S); Palomar Creek & Arroyo Seco, Santa Lucia Mts., (S).

The following specimens are slightly atypical and approach variety *dasyantha* in being less lanate and somewhat more branched. San Benito Co., Pinnacles National Monument, *Craig* 1370 (P), *Sutcliffe* in 1920 (C.A.). Ventura Co., Matilija Canyon, *Hoffmann* (S.B.).

Instead of being a widespread and abundant form as it is commonly regarded, *G. virgata* var. *typica* is a very localized race from Monterey Co., and is not common.

2b. *Gilia virgata* var. *dasyantha* (Brand) n. comb. *Navarretia virgata* var. *dasyantha* Brand, Pflanzenreich IV, 250:168. 1907; *Hugelia virgata* var. *dasyantha* (Brand) Jepson, Man. Fl. Pls. Calif., 793, 1925.

Plant 5–35 cm. high, white floccose, glabrate in age, usually much branched both from base and nodes, leaves linear or divided into 3 linear lobes ascending or reflexed, flower clusters from 5—over a hundred, 3–10-flowered; bracts usually 6–14, commonly 8 mm. long; flowers regular or very slightly irregular, blue 14 mm. long; lobes almost $6\frac{1}{2}$ mm. long less than one third as broad; stamens yellowish, 5 mm. long, attached about 1 mm. from sinuses; anthers $1\frac{1}{2}$ mm. long, linear-sagittate.

Type, I have been unable to ascertain the type of this variety. Brand cites Hall 98 as the type, but I have been unable to locate this collection, unless the Hall collection from Riverside, July 3, 1897 (C) is this collection. San Bernardino, *Parish*, 1478 (C); *Parish* 3803 (C); So. Calif., *Parry* and *Lemmon* 249 (P) are all cited by Brand as “typical” *dasyantha*. These show beyond reasonable doubt to which plants *dasyantha* should refer. Ranging throughout cismontane Southern California south of Santa Barbara County. Representative material studied, CALIFORNIA: San Diego Co., San Onofre Canyon, *Hitchcock* in 1929 (P). Orange Co., Trabuco Canyon, *Abrams* 1791 (P); Rancho Santa Ana, *J. T. Howell* 73 (S.A.). Riverside Co., Wilder’s near Riverside, *Wilder* 45 (C); Temecula, *Jones* 1926 (P); Riverside, *Hall* in 1897 (C). Ventura Co., Santa Susanna Pass, *J. T. Howell* 1020 (S.A.). Santa Barbara Co., Hot Springs, *Dudley* in 1896 (S). Los Angeles Co., Santa Monica Mts., *J. Ewan* 3480 (P); Downey, *Zumbro* 58 (P); San Fernando Valley, *Barber* 168 (P, C); Los Alisos Canyon, *Epling* and *Dunn*, in 1931 (C); Pomona, *Chandler* in 1897 (C); Sierra Madre, *Abrams* 2636 (P). San Bernardino Co., Plains near San Bernardino, *Parish* 11,888 (C); Colton, *Braunton*, 475 (C), *Pringle* in 1882 (P); San Bernardino, *Parish* in 1900 (P); Dry Mesas, San Bernardino Valley, *Parish* 11,376 (C), albino plant; Near Bloomington, *Parish* 11,282 (C), Cucamonga, *Abrams* 2661 (P); Claremont, *Craig* 1860 (P), *Munz* 2240 (P), *Baker* 3345 (C, P), *W. R. Shaw* in 1900, albino plant, *Craig* 529 (P), dated Nov. 24, 1927—blooming into the second season, *Munz*, 4404 (P), dated Dec. 5, 1920—growing into the second season.

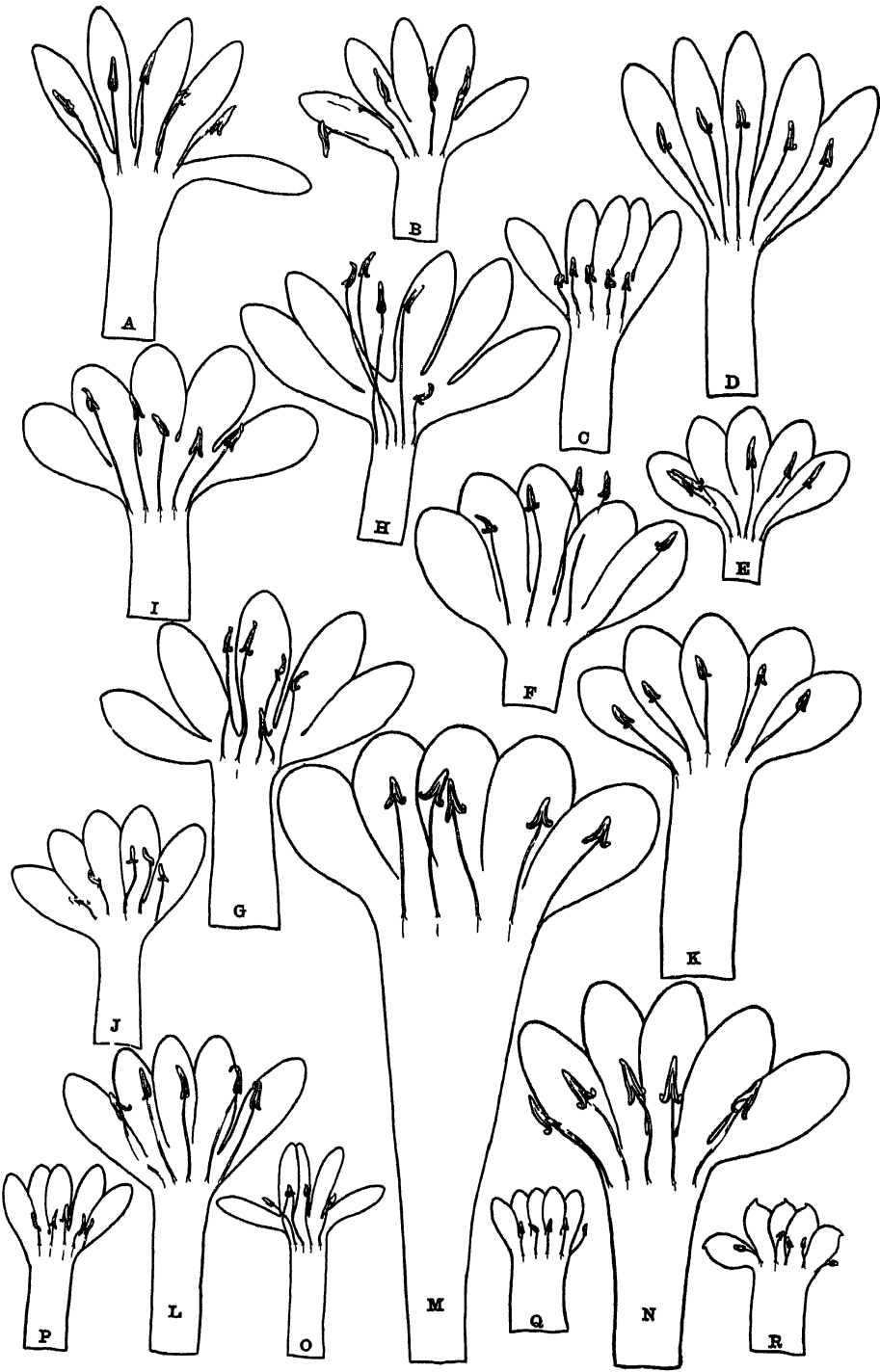
Var. *dasyantha* is the commonest variety of *G. virgata*. It differs from var. *typica* in being much more branched, slightly smaller, more slender flowered, in having smaller but more numerous flower clusters. It is highly variable and intergrades to some extent with all the other varieties of the species.

The following collections represent a race of var. *dasyantha* differing from typical form in the coarseness of its growth. Riverside Co., Cabazon, *Munz* 898 (P). San Bernardino Co., Mentone, *R. F. Smith* 81 (C).

(To be concluded)

Explanation of plate 23

Fig. A. *G. pluriflora*—Lemon Cove, Tulare Co., Cal., *Condit* in May (P.) Fig. B. *G. lutescens*—Santa Ana Mts., Cal., *Munz* 7103 (P.) Fig. C. *G. Wilcoxii*—10 mi. n. e. of Reno, Nev., *Munz* 11,100 (P.) Fig. D. *G. virgata* var. *dasyantha*—San Bernardino Valley, Cal., *Parish* 11,281 (P.) Fig. E. *G. virgata* var. *ambigua*—Wrightwood, San Gabriel Mts., Cal., *Munz* and *Johnston* 11,197 (P.) Fig. F. *G. virgata* var. *sapphirina*—North base of Sugar Loaf Mt., San Bernardino Co., Cal., *Munz* 10,760 (P.) Fig. G. *G. eremica* var. *typica*—2½ mi. east of Barstow, Cal., *Wolf* 3390 (S.A.) Fig. H. *G. eremica* var. *zionis*—Zion National Park, Utah, *Craig* 1418 (P.) Fig. I. *G. eremica* var. *arizonica*—Wickenburg, Ariz., *Jones* in 1903 (P.) Fig. J. *G. eremica* var. *Yageri*—Yagers north of Tucson, Ariz., *Jones* in 1890 (P.) Fig. K. *G. Sherman-hoytiae*—12 mi. so. of Muroc, Cal., *Peirson* 7268 (P.) Fig. L. *G. densifolia* var. *mohavensis*—between Rosamond and Mohave, Kern Co., Cal., *Craig* 1360 (P.) Fig. M. *G. densifolia* var. *sanctora*—Santa Ana River Wash, Cal., *Reed* 3107 (P.) Fig. N. *G. densifolia* var. *austroromontana*—San Jacinto Mts. Cal., *Munz* and *Johnston* 8837 (P.) Fig. O. *G. filifolia* var. *typica*—San Diego, Cal., *Brandegee* in 1906 (C.) Fig. P. *G. filifolia* var. *sparsiflora*—Cactus Flats, San Bernardino Co., Cal., *Munz* 10,500 (P.) Fig. Q. *G. filifolia* var. *diffusa*—Colorado Desert, Calif., *Munz* 11,921 (P.) Fig. R. *G. filifolia* var. *Harwoodii*—So. of Rice, Riverside Co., Cal., *Wolf* 3119 (S.A.).



CRAIG HUGELIA

INDEX TO AMERICAN BOTANICAL LITERATURE 1930-1934

The aim of this Index is to include all current botanical literature written by Americans, published in America, or based upon American material; the word America being used in the broadest sense.

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(CONCLUDED)

2c. *Gilia virgata* var. *sapphirina* (Eastwood) Macbride, Contrib. Gray Herb., n.s., 49: 58. 1917. *Gilia sapphirina* Eastwood, Bot. Gaz., 38: 71. 1904; *Navarretia virgata* var. *sapphirina* (Eastwood) Brand, Pflanzenreich IV, 250: 165; *Hugelia virgata* var. *sapphirina* (Eastwood) Jepson, Man. Fl. Pls. Calif., 793. 1925; *Navarretia virgata* Sub. Sp. *gymnocephala* Brand l.c., in large part; *Navarretia virgata* var. *oligantha* Brand l.c.

Plants erect 7–35 cm. tall, loosely paniculately branched from base; branches slender, sparsely leaved, commonly viscid-glandular; leaves 1–5 cm. long, commonly simple and sub-terete, the uppermost occasionally with 2 very short bristle-tipped divisions at base; flowers solitary, sessile, or pedicellate or borne in 2–4-flowered clusters; bracts broadly ovate and 3-lobed or membranous on either side of broad green rib, thinly pilose and commonly somewhat glandular, 3–8 mm. long; calyx 8 mm. long, campanulate, or tubular, glandular puberulent and viscid, not at all lanate; corolla regular to slightly irregular, commonly about 12 mm. long; lobes sapphire blue, elliptical 6–7½ mm. long, rarely twice as long as tube; tube yellow 4 mm. long; stamens 7–8 mm. long; anthers oblong, sagittate 2–2½ mm. long.

Type locality, San Jacinto Mts., Riverside Co., California. Representative material studied, CALIFORNIA: San Bernardino Co., Sugar Loaf Mt., *Munz* 10,760 (P); Santa Ana River 6500 ft., *Munz* 6157 (P); Bear Valley, *Pierce* in 1922 (P); Big Meadows, *Feudge* 1216 (P); Mission and Fish Creek divide, *Munz* and *Johnston* 8526 (P), Santa Ana River 8100 ft., *Munz* and *Johnston* 8633 (P). Los Angeles Co., Swartout Canyon, *Hall*, 299 (C). Riverside Co., Strawberry Valley, *Hall* 329 (C); San Jacinto Mts., *Hall* 2635 (C, P), *Trask* in 1903 (C.A.); Strawberry Valley, *Hoffmann* in 1929 (C.A.); Idylwild, *Spencer* 1657 (P); Hemet Valley, *Munz* 5973 (P); Hemet, *Wilder* 959 (C) approaches *dasyantha* in size of flower clusters. San Diego Co., Palomar Mt., *Meyer*, 489 (C), *T. S. Brandegee* in 1898 (C); Witch Creek, *Alderman* 401 (C), Laguna Woods, *Cleveland* in 1885 (C); Cuyamaca Mts., *K. Brandegee* in 1906 (C, P), *K. Brandegee* in 1874 (C); Buckman Springs, *Meyer* 434 (C); Descanso, *Spencer* 918 (P); Viejas, *K. Brandegee* in 1906 (C); Warner's Hot Springs, *Buttle* in 1913 (C.A.); 10 mi. so. of Warner's Hot Springs, *J. T. Howell* 3268 (C.A.). LOWER CALIFORNIA: 9 mi. southeast of Tecate, *Munz* 9475 (P); 10 mi. north of Ojos Negros Rancho, *Wiggins* and *Gillespie* 4133 (C.A.).

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Vars. *sapphirina* and *ambigua* form quite a distinct section of *G. virgata*, having slightly irregular corollas, with longer stamens and shorter tube, and great reduction in the size of the flower clusters. Var. *sapphirina* as intended by Miss Eastwood and as I have applied the name, refers to the large flowered, glandular form found most commonly in the San Jacinto Mts. at elevations of 5000–8700 ft. Northward var. *sapphirina* ranges through the Mts. into San Bernardino and Los Angeles Counties, where it intergrades with var. *ambigua* Jones. The following collection is typical of this intergradation: Bear Valley, Jones 9917 (P). Southward var. *sapphirina* ranges through the higher Mts. into Northern Lower California. These most southern examples usually show an extreme variation in the reduction in the size of the flowers and flower clusters, for occasional plants have the flowers solitary and pedicelled. To these extreme examples Brand has given the name var. *oligantha*, I do not consider them distinct enough for recognition as a variety. The most extreme example of this reduction in the size of flowers and heads is Santa Catalina Mts., Lower Calif., Orcutt in 1883 (C), which is possibly type material of Brand's var. *oligantha*. I have seen two doubtful collections from the low coastal areas of San Diego County: Point Loma, Brandegee in 1906 (C); near San Diego, 1900 ft., Spencer 68 (C); but apparently var. *sapphirina* is an extreme rarity at elevations below 4000 ft.

Two other sheets from Lower California, Vallederos Creek, Brandegee in 1893 (C); Guadalupe Creek, Brandegee in 1893 (C), are of special interest, for apparently they represent still another race closely related to var. *sapphirina* and var. *ambigua*, and at the same time greatly resembling *eremica* in habit. The flower corresponds very closely to collections of var. *ambigua*, especially those from the high desert plateaus of San Bernardino County. I may cite Orcutt, July 9, 1884, from Hanson's Ranch northern Lower Calif., as a collection of var. *sapphirina* that resembles these sheets and that indicates a close relationship to that variety.

2d. *Gilia virgata* var. *ambigua* (Jones) n. comb.; *G. floccosa* var. *ambigua* Jones, Contrib. W. Bot. 13: 2. 1910.

Plant 10–25 mm. high, paniculately much branched, sub-glabrous to somewhat glandular; leaves 10–30 mm. long, linear and entire, or the uppermost with 2 spinose lobes at base; bracts broadly oval to orbicular 3–5-lobed; calyx 5 mm. long pilose to densely lanate, tubular; corolla pale, sapphire, or deep blue, 8–10 mm. long; lobes broadly elliptical 4–5 mm. long; stamen $4\frac{1}{2}$ – $5\frac{1}{2}$ mm. long, anthers white oblong sagittate, $1\frac{1}{2}$ –2 mm. long.

Type locality, Victorville (Victor), Mohave Desert, California. Representative material studied, CALIFORNIA: San Bernardino Co., 12 mi.

southeast of Victorville, *J. T. Howell* 108 (S.A.); Victorville, *Jones* in 1903 (type) (P); Mohave River district, *Palmer* 405 (C); southward from Barstow, *Jones* in 1926 (P); Box S Ranch and northward, *Jones* in 1929 (P); 2 mi. east of Deadman's Point, *Munz* 12,951 (P); Wrightwood, *Munz* and *Johnston* 11,197 (P). Los Angeles Co., 5 mi. south of Lancaster, *Munz* and *Craig* 12,953 (P); 12 mi. south of Muroc, *Munz* and *Craig* 12,926 (P); Monrovia Canyon, *Howell* 3879 (C); Lancaster, *K. Brandegee* in 1888 (C); San Antonio Mts., *Hall* 237 (C); Swartout Valley, *Munz* 7724 (P, C); Sheep Creek, *Peirson* 4128 (P); 3 mi. east of Vincent, Edge of San Gabriel Mts., *Craig* 2022 (P).

Var. *ambigua* is very much localized in the high desert plateaus and Mts. of Los Angeles and San Bernardino Counties, but there are several isolated collections from localities quite distant but ecologically similar. Inyo Co., Westgard Pass, *Hoffmann* (S.B.); Walker's Pass, *Purpus* 5391 (C). Kern Co., Buena Vista Hills, *Davidson* 580 (Ewan). Riverside Co., Aguanga, San Jacinto Mts., *Condit* in 1910. San Diego Co., Campo, *T. S. Brandegee* in 1904 (C).

Mr. Jones' use of the name *ambigua* in publication is somewhat different from his use of the name in his herbarium, which latter use is, of course, disregarded. The collection from Victor (Victorville) no. 10,011 cited first by him in the original description and consequently considered the type in this paper, is immature but representative of desert material and explains clearly the use of the name for publication. The collection from Bear Valley, San Bernardino Co., cited secondly, *Jones* 9917 is not at all typical being an intergrade near var. *sapphirina*.

3. *GILIA LUTESCENS* Steud., ex Benth., in D. C. Prodr. 9: 311. 1845. *Hugelia lutea* Benth., Bot. Reg. 19: sub. t. 1622. 1833. Not *G. lutea* (Benth.) Steud. *Navarretia lutea* (Benth.) Brand, Pflanzenreich IV, 250: 168. 1907; *Gilia floccosa* A. Gray, Proc. Am. Acad. 8: 272. 1870. (As to type.)

Plant annual, erect, 8–30 cm. high, few to many branched; stems glabrate; leaves usually narrowly linear 8–25 mm. long, rarely parted into 3 filiform segments; inflorescence white woolly 2–8-flowered; flowers bright sulphur yellow, 7–9½ mm. long, lobes almost equalling tube; stamens inserted in tube ¼ of distance below sinuses; ovules solitary in the cells.

Type locality, California, Douglas; probably near Monterey. Range, from Monterey Co. the species extends south through the hills and valleys of San Luis Obispo Co. across the north (desert) slopes of the San Gabriel Mts., to its southern limit on the east side of the Santa Ana Mts. It is very rare, at least in the southern part of its range. Representative material studied, CALIFORNIA: Monterey Co., California, Douglas (C) (probably

type collection); Nacimiento River and Gorda, *K. Brandegee* 136 (P, C); Jolon, *K. Brandegee* in 1909 (C), *T. S. Brandegee* (C). San Luis Obispo Co., Mts. north of San Luis Obispo, *Lemmon* in 1878 (C), *Lemmon* in 1896 (C), Los Angeles Co., Ravenna, *K. Brandegee* in 1889 (C), *K. Brandegee* 135 (C, P); Acton, *Hoffmann* (S.B.). Riverside Co., Santa Ana Mts., *Munz* 7103 (P, C).

G. lutescens is very closely related to *G. virgata*, especially to var. *ambigua* with which it may intergrade in Southern Calif. Because those who have been fortunate enough to see it in the field, regard it as a distinct species, I have chosen to regard it as such. As possible intergrades I cite the following collections: Temescal Canyon, *Peirson* 4707 (F.P.); Near Glen Ivy, *Howell* 1044 (S.A.); Roadside, Mt. San Jacinto, *Spencer* 1191 (C.A.); Slope of Mt. San Jacinto, *Spencer* in 1919 (P).

4. *GILIA PLURIFLORA* Heller, Muhl. 2: 113. 1906. *Gilia virgata* var. *floribunda* A. Gray, Proc. Am. Acad., 8: 272. 1870 (Not *Gilia floribunda* Gray). *Navarretia virgata* var. *floribunda* (Gray) Brand, Pflanzenreich IV, 250: 168. 1907; *Gilia Braunttonii*, J. & M., Jepson, Ec. Pls. Calif., 130. 1924; *Hugelia Braunttonii* (J. & M.) Jepson, Man. Fl. Pls. Calif., 793. 1925.

Plant annual, 12–35 cm. high; stem rarely simple, commonly corymbose branched; leaves sessile 15–50 mm. long, pinnately parted into 3–7 filiform divisions; flower clusters densely floccose; large, 15–40 mm. broad, 8–50-flowered; corolla well exserted, vivid blue to violet, salverform, 13–18 mm. long, lobes two thirds as long as tube; stamens 4 mm. long, attached at sinuses, exserted.

Type locality, Sunset, Kern Co., Calif. Range, this species is found in the hot interior valleys of Calif. from Alameda Co. to southern Kern Co. In Tulare and Kern Counties the species reaches its maximum development in size of heads and in length of the filiform leaves. Representative material studied, CALIFORNIA: Alameda Co., Corral Hollow, Geologic Survey Calif. no. 1212 *Brewer* (C). Monterey Co., Coburn Mill, *T. S. Brandegee* in 1879 (C). Mariposa Co., Yosemite, *Dodd* in 1891 (C). Fresno Co., Coalinga, *Condit* in 1910 (C). Tulare Co., Badger, *Brandegee* in 1892 (C); Middle Tule River, *Dudley* 876; Lemon Cove, *Condit* May 20 (P); South Fork of Kaweah River, *Culbertson* in 1904 (P, C); Kaweah River Valley, *Coville* and *Funston* 1318 (S); above Coffee Pot Canyon, El. 9000 ft. Sequoia National Forest, *Dudley* 1784 (S). San Luis Obispo Co., Estrella, *Jared* in 1897 (C). Kern Co., Bakersfield, *Davy* 1730 (C); north slope Tehachapi, *Munz* 11,428 (P); Sunset, *Heller* 7734 (C, S); Cameron, *Brandegee* in 1884 (C); Fort Tejon, *Parish* 1897 (S). Santa Barbara Co., 55 mi. east of Santa Maria, *Munz* 11,416 (P, C).

The following specimens approach *G. virgata*. Monterey Co., Cholame Valley, *Lemmon* in 1878 (C); *Congdon* in 1893 (P, C). Los Angeles Co., road from Palmdale to Victorville, *Peirson* 923 (F.P.).

Although the species has frequently been considered as merely a variety of *G. virgata*, it should, I believe, be considered distinct because the species are so strikingly different, and evidences of intergradation are not great for entities within such a variable group.

5. *Gilia Sherman-Hoytiae* Craig n. sp.

Erect annual 6–12 cm. high, much branched; stems slender, dark red brown; flower clusters 7–20, with 2–8 flowers to a cluster; corolla well exerted, $14\frac{1}{2}$ –17 mm. long, commonly regular or very rarely but slightly irregular with the dominant color a pale but vivid coerulean blue, very rarely white or pale lavender; lobes 5 mm. long, more than half as broad, pale, vivid coerulean blue, or very rarely with one or two light pencilings at base; tube 12 mm. long, at least twice as long as lobes, the upper third orange yellow, the lower third purple; stamens 4 mm. or less in length, attached at or near sinuses; anthers $1-1\frac{1}{2}$ mm. long. (Annuua, erecta, 6–12 cm. alta, ramosa; caulibus tenuibus; capitulis 4–20, cum 2–8 floris; corollis exsertis, 17 mm. longis, regularibus, coeruleis; lobis corollarum 5 mm. longis, ca. 3 mm. latis; tubo 12 mm. longo; staminibus 3–4 mm. longis; antheris $1-1\frac{1}{2}$ mm. longis.)

Type, Sandy Flats 10 mi. south of Muroc, Los Angeles County, California, *Munz* and *Craig* 12,925, Pomona College Herbarium no. 185,022. Isotypes widely distributed. Range, all the collections of this species I have seen are from the Antelope Valley, in Los Angeles and Kern Counties, Calif. Representative material studied, CALIFORNIA: 12 mi. south of Muroc, *Munz* and *Craig* 12,930 (P), *Peirson* 7268 (P, F.P.); between Redman and Muroc, *Hoffmann* in 1931 (C.A.), *Hoffmann* (S.B.); Muroc, *Hoffmann* (S.B.); Lancaster, *Davy* 2278 (C), *K. Brandegees* in 1909 and 1910 (C); 16 mi. east of Lancaster, *Munz* and *Craig* 12,952 (P).

This species is very local, for the only place it is known to grow is in the Antelope Valley region near Lancaster and Muroc, where in favorable seasons it abounds in the low sandy flats and sand dune areas forming large masses of vivid coerulean blue. One collection, Maricopa Hills, Kern Co., *Eastwood* 3272 (C.A.), which I believe, represents an intermediate form between *Sherman-Hoytiae* and *pluriflora* is the only collection possibly referable to this species which I have seen that does not come from within a radius of 20 miles from the type locality.

Superficially this plant resembles *G. eremica*, but the flowers are regular, the stamens short and attached near the sinuses, the anthers are very small and similar to those of *G. filifolia*, the corolla lobes are short as in *G. filifolia* but the flower averages more than twice as long as *G. filifolia* and is

correspondingly large, being well exserted, in this respect resembling *G. pluriflora*. Because of these characters, it is difficult to state to which of these species it is most closely related, but the general flower structure, especially the stamens and anthers, would indicate a close relationship to *G. filifolia*, in spite of the fact that superficially it resembles more closely either of the other species. This species is named in honor of Mrs. Albert Sherman Hoyt, who has contributed much to the Botany of the Southwest through her work to preserve the flora of the deserts.

6. *Gilia eremica* (Jepson) n. comb. *Hugelia eremica* Jepson, Man. Fl. Pls. Calif., 793. 1925; *Gilia floccosa* Gray, Proc. Am. Acad. 8: 272. 1870, in part, not as to type. *Hugelia floccosa*, Nutt., ex. Gray, l.c. *Gilia virgata* var. *floccosa* (Gray) Milliken, Univ. Calif. Pub. Bot. 2: 40. 1904, in part; *Navarretia virgata* subsp. *floccosa* Brand, Pflanzenreich IV, 250: 168. 1904, in part; *Welwitschia floccosa* Rydb., Fl. Rocky Mts., 688, 1065. 1917, in part.

Plant annual, 3–25 cm. high, floccose, glabrate in age, rarely somewhat glandular or sub-glabrous; stem commonly much branched from base or rarely almost simple; leaves 8–45 mm. long; flower clusters 2–more than 100, lanate, 2–10-flowered; bracts 3–7-lobed; flowers well exserted, commonly strongly two lipped, or rarely almost regular, white, lavender, or blue, variously marked; from 2–8½ mm. long; attached at sinuses or in the tube; anthers from less than 1 to 2 mm. long.

This use of the name *G. eremica* is much more inclusive than that of Jepson, and there are several good reasons for this usage. *G. eremica* var. *typica* is much more common and widespread than hitherto recognized. The species is a highly variable one and runs into a number of forms with corollas of variable irregularity. These varieties again show indications of intergradation with other species. These intergradations have been discussed under *G. Sherman-Hoytae*, *G. virgata* vars. *sapphirina* and *ambigua* and will be discussed under *G. filifolia* var. *diffusa*. Most of these have been referred to *G. floccosa* a name which must be placed in synonymy because of its original use by Gray as a new name for *G. lutescens* Steud. (*G. lutea* Benth.). This name, *floccosa*, has been applied to many forms of at least two or three species. Because of the uncertainty of this later usage it is obviously desirable to drop the name in accordance with accepted rules of nomenclature.

KEY TO VARIETIES

- A. Leaves with from 5–9 linear lobes, flowers markedly irregular. Deserts of So. Calif. and southwest Nevada.

6a. *Gilia eremica* var. *typica*.

- AA. Leaves commonly linear or 3-lobed, rarely 5-lobed, Arizona, Utah, Nevada.

B. Corolla 14 mm. long or longer; stamens 3–8½ mm. long, the anthers more than 1 mm.

C. Corolla markedly irregular; the lobes two thirds the length of tube, more than 2 times as long as broad; longest stamen more than 3 times longer than shortest stamen; never glandular. High elevations, Utah, So. Nevada, Northern Arizona.

6b. *G. eremica* var. *zionis*.

CC. Corolla slightly irregular; the lobes about ½ length of tube, the lobes less than 2 times as long as broad; longest stamen about 2 times longer than shortest stamen. Sometimes glandular.

6c. *G. eremica* var. *arizonica*.

BB. Corolla less than 13 mm. long, very slightly irregular; the stamens 2–3 mm. long, the anthers less than 1 mm. long. Southern Arizona.

6d. *G. eremica* var. *Yageri*.

6a. *Gilia eremica* (Jepson) var. *typica* n. nom. *Hugelia eremica* Jepson, Man. Fl. Pls. Calif. 793. 1925; *Navarretia densifolia* var. *jacumbana* Brand, Ann. Conserv. et Jard. Bot. Genève, 15 & 16: 340. 1913.

Plant commonly floccose, or rarely glabrate, or sub-glabrous, much branched from base 8–20 cm. high; leaves usually recurved, 1–4 cm. long, oblong in outline with from 5–9 linear lobes; the lobes 2–8 mm. long; flower clusters 5–more than 100, lanate, 3–10-flowered; bracts 3–7-lobed; flowers well exserted, bilabiate rarely white, more commonly pale lavender, unmarked, or with the upper 3 lobes with red violet markings at base, the lower 2 lobes pale lavender and unmarked; corolla 16 mm. long; lobes 6 mm. long; tube 10 mm. long, variably marked with yellow and lavender; stamens attached about 1 mm. below sinuses and of variable length from 2–6 mm. in length, a common arrangement being 2 excurbent and short, and 3 incumbent and long, or 1 short, 2 medium and 2 long, always with at least one stamen well exserted; anthers 1½–2 mm. long.

Type locality, Calico Wash northeast of Barstow, Mohave Desert, California. Representative material studied, CALIFORNIA: Colorado Desert, San Bernardino Co., Morongo Pass, *Armacost* in 1928 (P); Morongo Valley, *J. T. Howell* 536 (S.A.). Colorado Desert, Riverside Co., Morongo Wash, *Gilman*, a15 (P); Whitewater, *Jones* in 1926 (P), *Munz* 4552 (P); Coachella Valley, *Gilman* in 1928 (P); Palm Springs, *Munz* and *Harwood* 3526 (C, P), *Jones* in 1903 (P); Van Deventer's, *Hall* and *Jepson*, 1892. Colorado Desert, San Diego Co., Borego Valley, *Munz* and *Hitchcock* 11,336 (P); Sentenac Canyon, *Munz* and *Hitchcock* 11,365 (P); Palm Creek, *T. S. Brandegee* in 1895 (C). Mohave Desert, San Bernardino Co., 2½ mi.

east of Barstow, *Wolf* 3390 (S.A.); Calico Wash N.E. of Barstow, *Jepson* 5414 (Herb. Jeps. Type); Daggett, *Hall* 6142 (C); Barstow, *K. Brandegee* in 1909 (P); Helendale, *Craig* 1390 (P); Kramer, *K. Brandegee* in 1912 (P); 3 mi. east of Deadman's Point, *Munz* 12,950 (P); Box S Ranch, *Munz* and *Hilchcock* 12,172 (P, C), *Jones* in 1926 (P); Adclanto, *S. B. Parish* 11,806 (C); Cave Spring, Old Dad Mt., *Jones* in 1926 (P); Providence Mts., *Munz*, *Johnston* and *Harwood* 4280 (P); Old Woman Mts., *Jones* in 1926 (P); Fenner, *Munz*, *Johnston* and *Harwood* 4175 (P). Mohave Desert, Kern Co., Indian Wells Valley, *F. Peirson* in 1927 (F.P.); Bissell, *K. Brandegee* in 1912 (P); Mohave, *Jones* in 1884 (P), *Jones* in 1903 (P); Walkers Pass, *Purpus* 5391 (C); Argus Mts., *Purpus* 5421 (C). NEVADA: Canyon Station, Clark Co., *Heller* 10,435 (C).

G. eremica var. *typica* is a very common plant in the southern Mohave Desert and on the northern Colorado Desert. In this area, the center of its range, it is a very distinct and stable form, but along the borders of its range it intergrades freely with other forms.

The following collections from along the eastern slopes of San Jacinto and other principal dividing ranges to the southward seem to show a definite tendency toward smaller, darker, more regular flowers. They remotely approach the irregular flowered forms of *virgata*. San Bernardino Co., Banning, *Gilman* 21 (C). San Diego Co., San Felipe Valley, *Keck & McCully*, 66 (P), *K. Brandegee* in 1899 (P); Desert slopes, Jacumba, *Abrams* 3640 (P, C). This is the type collection of *G. densifolia* var. *jacumbana* Brand; San Jacinto Mt., 5000 ft., *Spencer* 1018; eastern base of San Jacinto Mts., *Hall* 2119 (C).

To the east var. *typica* seems to intergrade with var. *zionis*. ARIZONA: Ft. Mohave, *Lemmon* in 1884 (C). NEVADA: Las Vegas, *Craig* 1462 (P); Bunkerville, *Jones* in 1923 (P). CALIFORNIA: Cima, *K. Brandegee* in 1915 (C). *G. eremica* var. *typica* is a plant of the lower altitudes while var. *zionis* is from higher altitudes.

6b. *Gilia eremica* var. *zionis* Craig n. var.

Plant 6–25 mm. high; leaves 8–30 mm. long, linear or 3-lobed, rarely 5-lobed; the lobes arising from the basal third of rachis; corolla irregular but less so than in *typica*, 14 mm. long; the lobes $6\frac{1}{2}$ mm. long less than $\frac{1}{2}$ as wide, commonly blue or tinged with lavender; tube 8 mm. long, variously marked; stamens attached in tube about 2 mm. below sinuses of variable length, a common arrangement being one 2 mm. long, two 7 mm. long, and two $8\frac{1}{2}$ mm. long. (Planta 6–25 mm. alta; foliis 8–30 mm. longis, linearibus vel 3-lobatis, lobis corollarum $6\frac{1}{2}$ mm. longis, $2\frac{1}{4}$ –3 mm. latis, azureis, tubo 8 mm. longo; staminibus in tubo adnatis, ca. 2 mm. infra sinos.)

Type, Zion National Park, Utah, June 18, 1928, *Craig* no. 1400, Pomona College Herbarium no. 184,135, isotype Pomona College Herbarium no. 182,268. Representative material studied, UTAH: Zion National Park, *Craig* 1402 (P), *Craig* 1418 (P); Beaver Dam Mts., *Craig* 1394 (P); between St. George and Las Vegas, *Goodman* and *C. L. Hitchcock* 1665 (C); La Verken, *Jones* 5194 (C, P), *Jones* 5189 (P). ARIZONA: Burnt Canyon, Coconino Co., *Cottom* 4198 (P); Agua Caliente, *Carlson* in 1914 (C.A.); road from Chloride to River, *Eastwood* 15,326 (C.A.); Oatman, *Eastwood* 18,206 (C.A.); Kingman, *Eastwood* 18,026 (C.A.). NEVADA: base of Charleston Mts., *Tidestrom* 9659 (C.A.).

The following collections are intergrades very near to var. *zionis*, but approaching vars. *typica* and *arizonica*. UTAH: La Sal Mts., *Purpus* in 1899 (C). ARIZONA: Clifton, *Rusby* 269 (C); Yucca, *Jones* 9936 (P). CALIFORNIA: Panamint Valley, Inyo Co., *Parish* 10,162 (C); Barnwell, *Brandeggee* in 1911 (C); Darwin Mesa, *Hoffmann* (S.B.).

Var. *zionis* is the variety of *G. eremica* most closely related to var. *typica*; however, there seems to be very little intergradation between these two forms. The main differences between var. *typica* and var. *zionis* are that *zionis* is bluer and darker colored, it has a slightly less irregular corolla, the leaves are much less lobed, frequently being entire, when lobed, the lobes arise from the basal one third of the rachis.

6c. *Gilia eremica* var. *arizonica* Craig n. var.

Plant 4–20 mm. high, much branched; stems floccose-lanate or rarely glandular; leaves 10–45 mm. long, linear to 5-lobed, the lobes mostly basal; flower clusters loosely lanate; corolla 14–17 mm. long slightly irregular; lobes 5–6 mm. long, $\frac{1}{2}$ – $\frac{2}{3}$ as wide, blue; tube variously marked, the dominant color yellow; stamens attached about 2 mm. below sinuses, 3–6 mm. long. (Planta 4–20 mm. alta, multoramosa; caulibus floccoso-lanatis, rariter glandulosus; foliis 10–45 mm. longis, parce irregularibus, lobis 5–6 mm. longis, $2\frac{1}{2}$ –4 mm. latis, azureis; tubo luteo; staminibus 3–6 mm. longis.)

Type, Wickenburg, May 5, 1903, alt. 2100 ft., *Jones* 10,253, Pomona College Herbarium no. 74,569. Representative material studied, ARIZONA: Apache Trail, *Nelson* 6303 (C); Prescott, Phoenix highway, *Nelson* 10,263 (C); Roosevelt Dam, *Eastwood* 8686 (C.A.); Mazatzal Mts., *Eastwood* 16,937 (C.A.); Peach Springs, *Wilson* 145 (C); Franconia, *Jones* 1903 (P).

The following collections are small flowered forms representing intergrades with vars. *zionis* and *Yageri*. ARIZONA: Skull Valley, *Jones* 10,250 (P); Hillside, *Jones* 10,279 (P); Sierra Ancha, *Eastwood* 16,962 (C.A.); Globe, *Eastwood* 8654 (C.A.); Pinal Mt. on road to Winkleman, *Eastwood*, 17,318 (C.A.); Hualpi Indian Reservation, *Dudley* in 1931 (C.A.); Verde, *Wyatt Jones* in 1920 (C).

Var. *arizonica* is a large flowered form of *G. eremica* with a less irregular corolla and with short broad corolla lobes. It is found in the Lower Sonoran life zone in Arizona, most commonly in the southern part of the state. The collection by Mr. Jones, Wickenburg in 1903, which I have cited as the type, is marked by Mr. Jones as *Gilia virgata* var. *ambigua* type. Later he published this name for a different form from California and then cited this sheet as typical of var. *Yageri*. When selecting a type for var. *Yageri* from the long list of collections cited by him as typical, I have, on the advice of Dr. Munz, taken the collection from Yager's as the type sheet. Because this specimen from Wickenburg is most typical of var. *arizonica*, I am naming it the type of that variety. Nelson 10,303 and 10,263 are also extreme examples of the variety.

6d. *Gilia eremica* var. *Yageri* (Jones) n. comb. *G. virgata* var. *Yageri* Jones, Cont. W. Bot. 13: 2. 1910.

Plants 3–15 mm. high, lanate to sub-glabrous; leaves 7–20 mm. long, linear to 5-lobed which lobes arise from basal third of rachis; flower clusters 2–25, 2–5-flowered; corolla slightly irregular, 12 mm. long, lobes 5 mm. long, half as wide, pale blue; tube yellow; stamens 2–3 mm. long attached near sinuses; anthers 1 mm. long or less.

Type locality, Yager's north of Tucson, Arizona. Representative material studied, ARIZONA: Sacaton Mts., Pinal Co., *Peebles* and *Harrison* 1069 (P); Sacaton, *Peebles* 6515 (C.A.), *Eastwood* 8021 (C.A.); forty miles north of Tucson, *Jones* 25,672 (P); foot of Baboquivari Mts. 15 mi. northeast of Sells, *Fosberg* 7786 (P).

The following sheets are somewhat atypical but are more closely related to var. *Yageri* than to *G. filifolia* var. *diffusa*, the form to which they are most closely related. ARIZONA: mesa near Tucson, *Pringle* in 1883 (P); Tucson, *Eastwood* 8066 (C.A.), *Toumey* in 1894 (C); west side of Santa Catalina Mts., near Tucson, *Lemmon* 241 (C); Pinal Mt., *Eastwood* 17,318 (C); Hackberry, *Jones* 10,267 (P); *Jones* 10,269 (P); Wickenburg, *Jones* 10,265 (P); Phoenix, *Jones* 10,266 (P). MEXICO: 15 miles north of Magdalena, Sonora, *Fosberg* 7787 (P).

Var. *Yageri* is a very confusing form and I am uncertain to which species it should properly be referred, the flower is more regular than in any of the other varieties of *G. eremica* and the stamens are attached much closer to the sinuses and are short and with small anthers. These stamen characters indicate a relationship to *G. filifolia*, and in habit the plant closely approximates var. *diffusa* of that species, to which it is obviously very closely related. It is no doubt more closely related to both var.

diffusa and the Arizona varieties of *G. eremica* than to *G. virgata* the species to which it was originally referred.

In his description of var. *Yageri*, M. E. Jones referred to the variety a number of sheets from Arizona, Nevada, Utah, and California. The specimen first cited was his no. 10,250 from Skull Valley, Arizona which I here refer to var. *arizonica*. He himself wrote "I regard the types as Nos. 10,279 and 10,253," the former being from Hillside, Arizona and so imperfect a specimen as to be impossible of exact reference, while the latter I am using as the type of var. *arizonica*. In view of the fact that Mr. Jones cites also Utah material referable to var. *zionis*, and a California plant belonging to *G. eremica* var. *typica* it is evident that he included under his var. *Yageri* plants of four different entities. Since his use of the word "type" is not that of a single specimen, and since he used the name *Yageri* and cited a specimen from Yager's, it would seem to give the least confusion to re-typify var. *Yageri* and to designate as the type the plant from which he drew the name, i.e. Jones 9935, from Yager's, north of Tucson, Arizona, Pomona College Herbarium no. 74,576.

7. *Gilia filifolia* Nutt., Journ. Acad. Phil. n. s. 1: 56. 1848. *Gilia virgata* var. *filifolia* (Nutt.) Milliken, Univ. Calif. Pub. Bot. 2: 39. 1904; *Navarretia filifolia* (Nutt.) Brand, Pflanzenreich IV, 250: 167. 1907; *Navarretia filifolia* subsp. *eufilifolia* Brand, l.c.; *Eriastrum filifolium* (Nutt.) Wootton and Standley, Contrib. U. S. Nat. Herb. 16: 160. 1913; *Gilia floccosa* var. *filifolia* (Nutt.) Nels and Macbride, Bot. Gaz. 61: 35. 1916; *Welwitschia filifolia* Rydb., Fl. Rocky Mts. 688. 1065. 1917; *Hugelia filifolia* Jeps., Man. Fl. Pls. Calif. 792. 1925.

Plant annual; canescent-lanate to sub-glabrous; 3–30 cm. high; flowers 7–9 mm. long, blue to almost white or rarely yellowish or pinkish; lobes about half the length of the tube or less; stamens 1–2½ mm. long, not well exerted; the anthers cordate oval ½–1 mm. long.

Like *G. virgata* and *G. eremica*, *G. filifolia* is a species with a wide distribution and with numerous geographic races. The species is found from California to Texas, and from Lower California to Washington and Idaho. It is a complex group with considerable intergradation between the varieties and to some extent with other species. The smallness of the flowers adds to the difficulty of studying the group. Besides flower size the best taxonomic characters are the relative shortness of the corolla lobes, the shortness of the stamens, and the small size of the anthers. However *Wilcoxii* has all these latter structural characters, and *G. Sherman-Hoytiae* has relatively short lobes and stamens, but the anthers, though small (1–1½ mm.), are not as small as those of *G. filifolia* (1 mm. or less). *G. eremica* var.

Yageri, as mentioned in the discussion of that variety, has small anthers and resembles *G. filifolia* var. *diffusa* in respect to habit.

KEY TO VARIETIES

- A. Lobes of the bracts often exceeding 5 mm. in length; corolla limb blue; plant pilose to sub-glabrous. Plants of cismontane Calif. from Santa Barbara into Lower California.

7a. *Gilia filifolia* var. *typica*.

- AA. Lobes of the bracts rarely 4 mm., never exceeding 5 mm. long; corolla limb pale to almost white, or yellowish, or pinkish. Floccose becoming glabrate. Plants mainly of desert areas of the northwest, or very rarely in Mts. of southwest.

- B. Corolla lobes apiculate. Plants of the sand hills of eastern Riverside County and San Bernardino County.

7c. *G. filifolia* var. *Harwoodii*.

- BB. Corolla lobes not apiculate.

- C. Stems erect from base.

7d. *G. filifolia* var. *sparsiflora*.

- CC. Stems decumbent from base. Plants of the southwest deserts.

7b. *G. filifolia* var. *diffusa*.

- 7a. *Gilia filifolia* Nutt. var. *typica* n. nom. *Gilia filifolia* Nutt., l.c.

Plant pilose to sub-glabrous, 4–40 cm. high; stem simple and virgate or many branched from base or axils; leaves 3–35 mm. long, slender filiform or with two filiform lobes at base; flower clusters 3–15-flowered; larger bracts 12–20 mm. long, lanate; flowers 9 mm. long; lobes 3 mm. long 1 mm. wide, blue; tube twice length of lobes, yellow; stamens $2\frac{1}{2}$ mm. long attached to tube 1 mm. below sinuses; anthers cordate, oval, $\frac{1}{2}$ mm. long.

Type locality, said to be Santa Barbara, California. Representative material studied, CALIFORNIA: Santa Barbara Co., Blochman's Ranch, near Santa Maria, *Eastwood* 453 (C.A.). San Diego Co., 18 mi. north of Lakeside, *Craig* and *Zornes* 1856 (P); La Jolla, *Clements* 81 (C); Granite, Vicinity of San Diego, *Spencer* 66 (C); Potrero, *Abrams* 3724 (P); San Diego, *Brandege* in 1906 (C); Cuyamaca, *T. S. Brandege* in 1894 (C); between Escondido and Bonsall, *Craig* and *Zornes* 1857 (P).

The following specimens differ from the specimens from California in habit, being more branched and somewhat dwarfed. The flower structure is like *typica* and I feel they should be referred here. LOWER CALIFORNIA: Ryerson's Ranch, *Brandege* in 1893 (C); Lower Calif., *Bran-*

degee in 1893 (C); Tia Juana, *Orcutt* in 1883 (C); San Telmo, *T. S. Brandege* in 1893 (C); Ensenada, *Jones* in 1925 (P).

The following specimens closely approach var. *diffusa*; Llano de Santana, *T. S. Brandege* in 1889 (C); San Julio, *T. S. Brandege* in 1889 (C).

The following collections represent intergrades: Elizabeth Lake Canyon, *Hoffmann* (S.B.) represents a large flowered intergrade with *virgata*; Frazer Mt. Park, So. Kern Co., *Hoffman* (S.B.) represents an intergrade with *sparsiflora* or *Wilcoxii*.

G. filifolia var. *typica* is the coastal race of *G. filifolia* and is found from Southern California south into Lower California, being most common in San Diego Co. There are two quite distinct races within the variety, those from the northern part of the range being erect and virgate in habit, and appearing very much like a small flowered *G. virgata* var. *typica*. In Lower California the plant is decumbent from the base and diffusely branched. Because there is very much intergradation between these two races and because they are identical in flower structure, I have deemed it wise to group them under one name. Together they form a rather distinct group which intergrades with the other varieties through the Lower California plants. Although there are other characters, such as a tendency towards glabrousness, and relative darkness of flower color, the long filiform lobed bracts around the flower-clusters is the more constant and is the most obvious character.

It is through *G. filifolia* var. *typica* that the sub-genus *Hugelia* approaches *Navarretia*, for *Navarretia Abramsii* is not only superficially like *G. filifolia* but differs from it but slightly in flower structure having oval instead of sagittate anthers. It must be noted that *G. filifolia* var. *diffusa* frequently has oval anthers.

7b. *GILIA FILIFOLIA* var. *DIFFUSA* Gray, Proc. Am. Acad. 8: 272. 1870. *Navarretia filifolia* var. *diffusa* Brand, Pflanzenreich IV, 250: 167. 1907; *Welwitschia diffusa* Rydb., Fl. Rocky Mts., 688, 1065. 1917, in part. Probably *Hugelia virgata* var. *pygmaea* Jepson., Man. Fl. Pls. Calif., 793. 1925.

Stems decumbent from base diffusely branched, 3–15 cm. high, thinly pilose, glabrate in age; leaves 3–15 mm. long, simple, linear or divided into 3–5 linear lobes; flower clusters 5–more than 100, 3–20-flowered; longest bracts rarely 12 mm. long, commonly 8 mm., the lobes of the bracts linear, not filiform; calyx 4–5 mm. long lanate; flowers 7–8 mm. long, pale blue to white; corolla lobes 2½ mm. long; tube 8 mm. long; stamens 2–2½ mm. long; anthers about ½ mm. long, cordate, oval.

Type locality, "Fort Mohave and Nevada to New Mexico and the borders of Texas." Representative material studied, CALIFORNIA: San Diego Co., San Felipe Hill, *Jones* in 1906 (P); *K. Brandege* in 1899 (C).

Riverside Co., Morongo Wash, *Munz* 11,921 (P); Palm Canyon, *Wilder* 709 (P); Colorado Desert, *Hall* 5965 (C); Borego Spring, *Jones* in 1906 (P); Yaqui Well, *Eastwood* 2680 (C.A.). San Bernardino Co., Morongo Wash, *Munz* and *Johnston* 5169 (P, C); Cima, *K. Brandegee* in 1915 (P, C); Providence Mts., *T. S. Brandegee* in 1902 (C); Needles, *Jones* in 1884 (P, C); Hackberry Mt., *Wolf* 3265 (S.A.). Kern Co., Lancaster, *Hoffmann* in 1930 (C.A.), *K. Brandegee* in 1909 (C). NEVADA: Fort Mohave, *Lemmon* in 1884 (C). ARIZONA: Tucson Plains, *Lemmon* 170 and 173 (C); Tucson to Nogales, *Peebles* and *Harrison* 7039 (C.A.); Kingman, *Eastwood* 18,385 (C.A.); Peach Springs, *Wilson* 146 (C); Nogales, *T. S. Brandegee* in 1892 (C); Congress Junction, *Jones* in 1903 (P); Sonoita, *Harrison* 7183 (P); Yucca, *Jones* in 1884 (P). Pima Co., Continental, *Harrison* 6941 (P). Maricopa Co., Wittman, *Peebles*, *Kerney* and *Hastings* 6799 (P); Douglas, *Carlson* in 1915 (C.A.). NEW MEXICO: Silver City, *Eastwood* (C.A.); Bowie, *Eastwood* (C.A.); Organ Mts., *Wootton* in 1900 (P, C.); Lordsburg, *Jones* 25,671 (P). UTAH: Milford, *Jones* in 1880 (P, C). TEXAS: El Paso, *Jones* in 1884 (P); Fort Bliss, (C.A.); Fort Bliss, *Clemens*, in 1917 (P).

G. filifolia var. *diffusa* has a wide distribution, being found from Calif. to Texas. It is a difficult variety to understand for it varies greatly and apparently merges with *G. eremica* through var. *Yageri* of that species. In this area the two forms are identical in habit, but can be distinguished by a careful examination of the flowers. Var. *diffusa* has smaller flowers, commonly 8 or less mm. long while in var. *Yageri* they are commonly 10–13 or more mm. in length, never less than 9 mm. In var. *diffusa* the stamens are short and not exerted while in var. *Yageri* they are exerted. The anthers of var. *diffusa* are relatively broader. Northward var. *diffusa* shows indications of intergradation with var. *sparsiflora*. Collections from the far eastern part of its range, from Texas, New Mexico, and Utah, have the anthers almost round. One sheet, Mrs. Joseph Clemens' collection from Fort Bliss, Texas may represent a local race closely related to var. *diffusa*. Superficially the plant resembles desert collections of var. *sparsiflora*. In the deserts of Southern California var. *diffusa* is more readily identified; the source of confusion here is with var. *Harwoodii*, as discussed under that variety.

7c. *Gilia filifolia* var. *Harwoodii* n. var.

Plant erect, canescent-lanate, glabrate in age; leaves 5–25 mm. long; longest bracts 10–15 mm. long; corolla $7\frac{1}{2}$ mm. long; corolla lobes $2\frac{3}{4}$ mm. long, apiculate; stamens 1– $1\frac{1}{2}$ mm. long attached nearer the sinuses than in *diffusa*. (Planta erecta, canescenti-lanata, tarde glabrata; foliis 5–25 mm.

longis; bracteis longissimis 10–15 mm. longis; corollis $7\frac{1}{2}$ mm. longis; lobis $2\frac{3}{4}$ mm. longis, apiculatis; staminibus $1-1\frac{1}{2}$ mm. longis.)

Type, sandy desert 1200 ft., Blythe Junction, Riverside Co., California, *Munz* and *Harwood* 3589, April 2, 1920, Pomona College Herbarium no. 7622. Representative material studied, CALIFORNIA: Riverside Co., south of Rice, *Wolf* 3119 (S.A.). San Bernardino Co., Kelso, *Jones* in 1906 (P), *Brandegee* in 1915 (P, C).

Var. *Harwoodii* is a variety from the sand hills near Blythe and near Kelso, Calif. It is closely related to var. *diffusa* but differs in being more erect, more woolly, and in having the corolla lobes apiculate.

7d. *Gilia filifolia* var. *sparsiflora* (Eastwood) Macbride, Cont. Gray Herb. n.s. 49: 58. 1917. *Gilia sparsiflora* Eastwood Cal. Acad. Science, Series 3, vol. 2: 291. 1902; *Navarretia filifolia* subsp. *sparsiflora* Brand, Pflanzenreich IV, 250: 167. 1907; *Hugelia filifolia* var. *sparsiflora* Jepson, Man. Fl. Pls. Calif., 792. 1925.

Plant floccose, sometimes glabrate, erectly branching 10–30 cm. high; flower clusters 2–5-flowered; leaves linear or with a pair of short lobes at base; lobes at base of bracts pungent, rarely more than 4 mm. long; calyx 5–6 mm. long, corolla pale blue, almost white, or rarely pinkish $7-8\frac{1}{2}$ mm. long, corolla lobes 3 mm. long; stamens sagittate, linear to oblong.

Type locality, King's River Canyon, California. Representative material studied, OREGON: Gateway, Jefferson Co., *Abrams* 9594 (P); Bend, Crook Co., *E. Nelson* 861 (C, U. of Wyo.); Anderson Valley, *Leiberg* 2385 (C); Desert Well near Button Springs, *Leiberg* 387 (C, P); Burnes, Harney Co., *Henderson* 8930 (C.A.); Base of Steins Mt., *T. Howell* 1885 (S). CALIFORNIA: El Dorado Co., Fallen Leaf Lake near Tahoe, *Eastwood* 7859; Lakeside Park, *Geis* 107 (C). Lake Co., Glenbrook, *Jusset* in 1928 (C.A.). Sierra Co., Loyalton, *Eastwood* 7859 (C.A.). Lassen Co., Eagle Lake, *M. S. Baker* and *Nutting* in 1894 (C); South side Rixey Mts., *M. S. Baker* and *Nutting* in 1894 (C). Kern Co., Near Isabella, *Hoffmann* (S.B.). Mono Co., Coville, *Jones* in 1929 (P). Fresno Co., South Fork of King's River, *Eastwood* in 1899 (C.A.) Cited as typical material by Miss Eastwood. Ventura Co., Frazer Borax Mine, *Abrams* and *McGregor* 199 (S); Lockwood Valley, *Hoffmann* 1552 (S.B.), *Dudley* and *Lamb*, 4685 (S); Mt. Pinos, *Hall* 6580 (C), *Hoffmann* (S.B.). San Bernardino Co., Morongo King Mine, *Parish* 3329 (S); Cactus Flats, *Munz* 10,500 (P, C). LOWER CALIFORNIA: Valley of Palms, *Jones* 9911 (P). NEVADA: Ormsby Co., Eagle Valley, *Baker* 1403 (C, P), *Baker* 11,435 (P); Carson City, *Jones* in 1897 (P), *Jones* 9910 (P) in part; Empire City, *Jones* 3968 (P); *Jones* 3969 (P) in part, Washoe Co., Franktown, *Jones* in 1882 (P); Washoe,

Heller 10,603a (C); Reno, *Hillman* in 1893 (C), *Munz* 11,101 (P); Franktown, *K. Brandegee* in 1911 (C); Verdi, *K. Brandegee* in 1911 (C). Humboldt Co., Big Creek Ranch, *Taylor and Richardson* 49 (P). White Pine Co. Shellborne, *Jones* in 1891 (P). IDAHO: Picaba, Blaine Co., *Macbride and Payson* 2984 (C).

Var. *sparsiflora* was originally described by Miss Eastwood from specimens collected in the Sierra Nevada. These represent a rare race of the variety, for there are two poorly defined races. The first from montane and cismontane California of which Miss Eastwood's collections are typical; as is *Hall* 6580 from Mt. Pinos (P). The other race is from desert regions of California, Oregon, Washington, Nevada and Idaho, and is more woolly and has larger flower clusters; *Munz* 11,101 is typical of this race.

Var. *sparsiflora* is very close to *G. Wilcoxii* and intergrades with that species. As examples of this intergradation I list the following collections, CALIFORNIA: Boca, Nevada Co., *Sonne* in 1888 (C). WASHINGTON: Crab and Wilson Creeks, Douglas Co., *Sandberg and Leiberg* 246 (C); Washington territory, *Canby* 1883 (C). OREGON: Devine Ranch, *Leiberg*, 2408 (C). IDAHO: Nampa, Canyon Co., *Macbride* 1069 (C); King Hill, Elmore Co., *Nelson and Macbride* 1093 (C); Challis, Custer Co., *Macbride and Payson* (C). As possible intergrades with var. *typica*, I cite the following, CALIFORNIA: Humboldt Co., Hyampun River, *Chestnut and Drew* in 1888 (C). Lake Co., *K. Brandegee* (C.A., S).

8. *GILIA WILCOXII* A. Nels. Bot. Gaz. 34: 27. 1902. *Navarretia Wilcoxii* (A. Nels.) Brand, Pflanzenreich IV, 205: 165. 1907; *Welwitschia Wilcoxii* (A. Nels.) Rydb., Fl. Rocky Mts., 688, 1065. 1917, as to name. *Gilia floccosa* Gray, Proc. Am. Acad. 8: 272. 187 in part, not as to type.

Plant floccose, erectly branching, 8–25 mm. high; leaves commonly 5-lobed rarely 3-lobed, never entire; flower clusters blue to pale blue or rarely pinkish; 10–13 mm. long; lobes 4–5 mm. long; stamens commonly about 2 mm. long commonly inserted about 1 mm. below sinuses, anthers $\frac{1}{4}$ —1 mm. long.

Type locality, near St. Anthony, Idaho. Representative material studied, UTAH: Gold Hill, Deep Creek Mts., *Jones* in 1891 (P); Dutch Mt., Tooele Co., *Jones* 9915 (P), *Jones* in 1891 (P). CALIFORNIA: Kern Co., Between Coso Hot Springs and Coso Junction, *Ferris* 7450 (S). Inyo Co., Lone Pine, *Jones* 9932 (P); Chat, *Hillman* in 1897 (P); Wyando Creek near Deep Springs Valley, *Ferris* 1374 (S); Taboose Pass, *Peirson* in 1913 (F.P.); Hockett Trail, *Peirson* in 1911 (F.P.); Panamint Mts., *Coville and Funston* 2143 (S); Surprise Canyon near Panamint City, *Howell* 3897 (C.A.); Castle Peak 9000 ft., *Diel* 39 (P); Bishop Creek, *Jones* in 1926 (P); Black Canyon, White Mts., *Duran* 2681 (C); Owens Valley,

Horn 2850 (C); Inyo Co., *Austin* (C); Lone Pine, *Jones* in 1927 (P). Mono Co., Sherwin Grade, *Munz* 11,077 (P). Lassen Co., Honey Lake, *Brandege* in 1892 (C); Bloody Canyon, *Chestnut* and *Drew* 717-1886 (C); 10 mi. south of Amedec, *Jones* 9972 (P). NEVADA: Washoe, below Boca, *Jones* 9943 (P); 10 mi. northeast of Reno, *Munz* 11,100 (P); Empire City, *Jones* 3969a (P). Humboldt Co., Pine Forest Mts., *Taulor* and *Richardson* 50 (C). Ormsby Co., King's Canyon, *Baker* 1234 (C, P). Esmeralda Co., Candelaria, *Shockley* 242 (C); Carson City, *Jones* 9910 (P) in part; Winnemucca, *Jones* in 1881 (P). IDAHO: Canyon Co., Big Willow, *Macbride* 163 (Univ. Wyo.). Lemhi Co., Salmon, *Payson* 1779 (Univ. Wyo.). Washington Co., Weiser, *Jones* 9914 (P); Dry Soil, St. Anthony, *Merrill* and *Wilcox* 862 (U.S., Univ. Wyo.), *Merrill* and *Wilcox* 822 (U.S., Univ. Wyo.), *Merrill* and *Wilcox* 952 (U.S.). Blaine Co., Picabo, *Macbride* and *Payson* in 1916, 2984 (P). Canyon Co., Nampa, *Macbride* 1069 (P). Elmore Co., King Hill, *Nels.* and *Macb.* 1093 (P); Blackfoot, *Jones* in 1909 (P). Custer Co., Challis, *Macb.* and *Payson* 3213 (P). WASHINGTON: Wilson Creek, *Sandberg* and *Leiberg* 7161 (P).

Gilia Wilcoxii is a form very closely related to *G. filifolia* var. *sparsiflora* and it may merge with that species to some extent. It is so closely related that Macbride preferred to relegate *G. Wilcoxii* to synonymy, Cont. Gray Herb., n.s. 49: 58, 1917. Earlier, Nelson and Macbride Bot. Gaz. 61: 35. 1916, placed the name as a synonym of *G. floccosa*, but because of the uncertainty surrounding that name, it is difficult to know just what is Nelson's late understanding of *G. Wilcoxii*.

In 1928 Dr. P. A. Munz collected near Reno two distinct forms growing together without any indication of intergradation. In habit they were very much alike, but one form *G. Wilcoxii*, (Munz 11,100) had large bright blue flowers, and 3-5-parted leaves, while the other *G. filifolia* var. *sparsiflora* (Munz 11,101) had small pale blue flowers and entire or 3-parted leaves. Mr. Jones also collected these two forms in Empire City in 1882 and made a note of their growing together on this collection. These field observations show clearly that there are these two distinct forms in this area. In the herbarium material I have studied, I have found two sheets with both forms mounted together as the same collection; Carson City, *Jones* 9910 (P); Amedee, Calif., *Davy* 3408 (C). Although *G. Wilcoxii* and *G. filifolia* var. *sparsiflora* seem to be distinct, due to apparent intergradation, there are no characters that will in all cases serve to separate the two forms. Although var. *sparsiflora* usually has flowers about $7\frac{1}{2}$ mm. long, and mostly entire leaves, and *G. Wilcoxii* usually has flowers 11 or 12 mm. long and 5-parted leaves, there are many intermediate sheets to make identification difficult.

Some collections of *G. Wilcoxii* suggest *G. virgata* indicating close relationship to that species: Benton, Mono Co., Calif., *Hall* 10,647 (P). A sheet from the White Mts., Esmeralda Co., Nevada, *R. S. Ferris* 674 (C) is a unique sheet that remotely suggests *G. virgata*; the unusual appearance may be due to the alt. (10,500 ft.) at which it grew. Unfortunately the type sheets of *G. Wilcoxii* are examples of the small flowered race that are very close to var. *sparsiflora* in size of corolla and are among those sheets that must be looked upon as near intergrades between the forms.

SPECIES INCERTAE ET INQUIRENDÆ

Hugelia lanata Lindl. Jour. Lond. Hort. Soc. 3, p. 74, 1848.

Because the type is not available, because the description might refer to any of several forms, and because the type locality "America meridionali (Mexico)" is so indefinite; I am unable to definitely refer this name to any form.

POMONA COLLEGE

CLAREMONT, CALIF.

**The anatomy of the leaf of *Zeugites munroana*,
an anomalous grass**

RALPH W. MCCOY

(WITH PLATE 24)

A typical grass leaf consists essentially of two parts, a base and a blade. The former is developed into an amplexicaul sheath which may be open to the base, or closed and tubular for all or much of its length. The blade is bifacial and possesses one series of vascular bundles. A small ligular prolongation of the sheath at the insertion of the blade is also present in most species. In a few broad-leaved tropical grasses such as *Orthoclada*, *Pharus*, *Phyllorachis*, and *Zeugites*, however, a petiole-like stalk is inserted between the blade and sheath.

The parts of the grass leaf are variously interpreted by present day morphologists. Bugnon (1921) contends that the blade is equivalent to the leaf-base of the dicotyledon and that the sheath of the grass leaf is a new structure, having no equivalent in dicotyledonous plants. Arber (1918, 1923) upholds the phyllode theory which states that the blade and sheath of the grass leaf correspond to the petiole and base of the dicotyledonous leaf. The common theory, mentioned by Hitchcock (1922), is that the sheath, petiole, ligule, and blade of the grass leaf are homologous to the leaf-base, petiole, stipules, and blade, respectively, of the dicotyledonous leaf.

The interpretation of the petiole-like stalk, in those species in which it occurs, seems to be the thing of critical significance in comparing these theories. This structure is probably more definitely developed in *Zeugites* than in any other known genus of grasses; and, although its nature might be expected to throw some light on the general problem, it seems that no anatomical study of the leaf of this genus has ever been made. An abundance of good living material of *Zeugites munroana* Hemsl.¹ has made possible a study which has revealed some facts not previously reported.

Inasmuch as the word *petiole* is not a clearly defined anatomical term, it seems best to apply it to the structure in question, even though it may not be the homologue of the petiole of the leaf of dicotyledons. Its presence gives rise to the following questions: (a) Does the anatomy of the petiole differ in any way from that of the blade or sheath? (b) What relationship exists between the anomalous form of the leaf and its venation?

¹ The plants used in this study were grown in the University greenhouse from seeds collected by Dr. Paul Weatherwax near Antigua, Guatemala.

(c) How could all four parts, sheath, ligule, petiole, and blade, have come from a single primordium in the bud?

The literature on the anatomy of the grass leaf is extensive, but the petiole has received little attention. Pée-Laby (1898) was interested in the anatomical characteristics of the leaves of the Gramineae which would have taxonomic value and endeavored to group the grasses of France according to these characteristics. He summarized the work done before 1898, mentioning, among others, the studies of Palisot de Beauvois, Hingshausen, and Duval-Jouve. Beauvois (1812) supported Linnaeus' theory that all grass leaves have an analogous structure and are composed of the same parts. Hingshausen (1865) attempted to classify the Gramineae by the nervation of the leaves and was the first to detect the communication between the parallel veins. Duval-Jouve (1875) was interested in comparative anatomy for taxonomic purposes. Brandis (1907) discussed the structure of various bamboo leaves, but ignored the petiole which is well developed in a number of genera of this group. In Bugnon's comprehensive work (Bugnon, 1921) on the leaves of grasses only a few lines are devoted to the petiole, and they do not touch the significant problems involved.

Goebel (1905) summarized the work done on leaf development up to 1894, making special mention of Steinheil, Schleiden, Trécul, and Eichler. Steinheil (1837) concluded that a leaf grows from above downwards and that the oldest part is at the tip. In a compound leaf, however, the upper leaflets are the youngest. Schleiden (1843) held that the leaf shoots out from the axis, and that the tip is the oldest and the base the youngest. Trécul (1853) noted that the process of leaf-formation may be dissimilar in different plants. Some leaves were observed to be acropetal and others basipetal, while many developed from the middle both upwards and downwards. He stated that the leaf-sheath was the first to arise, but his error was corrected by Eichler (1861) who found that the sheath was formed by intercalary growth out of the base of the leaf.

Goebel contended that those "parts which have the earlier functions to perform appear the earliest." In general, the tip of the monocotyledonous leaf is the oldest and further growth is basipetal and intercalary. He regarded the petiole as nothing more than a narrowed or constricted portion of the lamina, and found that a definite relationship existed between conformation of the leaf and the course of its veins.

GENERAL CHARACTERISTICS OF THE LEAF

The foliage leaf of *Zeugites munroana* consists of blade, petiole, ligule, and sheath. The mature blade is 1.5 to 3 cm. long, and 5 to 10 mm. wide.

It is ovate-lanceolate and rounded at the base, and is separated from the open sheath by a slender petiole whose length is usually about one-fourth that of the blade.

The petiole exhibits marked longitudinal differentiation (Pl. 24, fig. 1). The middle portion is darker in color than either extremity and has a flattened adaxial surface. The distal end of the stalk is terete, and by curvature of this upper terete portion the blade is able to assume various positions with reference to environmental conditions. The proximal end is oval in cross-section and it also is occasionally flexed.

The ligule is an erect outgrowth from the inner surface of the sheath, and is continuous with the thin overlapping edges of the leaf-base. The continuity of the ligule and these thin edges, both of which are somewhat hirsute, gives a slight hooded effect to the upper end of the sheath.

The leaf is convolute in the bud (Pl. 24, fig. 2) but there is no definite relationship between the overlapping in the blade and that of the sheath. In other words, the blade of one particular leaf may be wrapped to the right while its sheath is wrapped to the left, or vice versa; or both may be coiled with the same edge inward.

Apart from the usual bulliform and guard cells, the cells of both the upper and lower epidermis are remarkably uniform. They consist of two classes of cells: (a) the elongated cells with undulating edges, which cover most of the leaf surface, and (b) the smaller polygonal cells with straight edges found at the basal region of the lamina.

VENATION

Different methods of preparing the whole leaf for study were tried, but only one of these proved successful. The best results were obtained by severing the petiole, flattening the sheath, and then treating the two parts separately. These were decolorized, stained, and cleared for examination at low magnification. After the chlorophyll had been removed with alcohol, the blade and sheath were stained in anilin safranin for twenty-four hours, destained and dehydrated with alcohol, changed to xylol and mounted in balsam.

The vascular system of the blade consists of a midrib, and usually six other longitudinal veins almost as large, together with numerous distinctly smaller ones. By diverging at the base and converging at the tip of the blade, these veins become arcuate (Pl. 24, fig. 3). The small nerves traverse the spaces between the large ones, varying in number from one to eight in each space in the middle portion of the blade. As the blade narrows toward the tip and base, the slender nerves anastomose and become fewer in number. Eight of them enter the petiole from the lamina. Numerous

conspicuous cross-veins complete the vascularization of the blade. The diverging and converging arcuate nerves, with the anastomosing cross-veins, cause this leaf blade to simulate the netted-veined leaves of dicotyledons.

Cross-sections of the blade show that each longitudinal nerve consists of a vascular bundle and two girders of sclerenchymatous fibers, one on the adaxial and the other on the abaxial side (fig. 4). These fibrous strands form bars of hard tissue which add strength and rigidity to the leaf. The bundle is enclosed in a sheath of two portions: an outer ring of large, clear, parenchymatous cells, and an inner sheath of thick-walled and strongly lignified cells. The outer sheath, however, is interrupted by the fibrous girder on the abaxial side of the leaf.

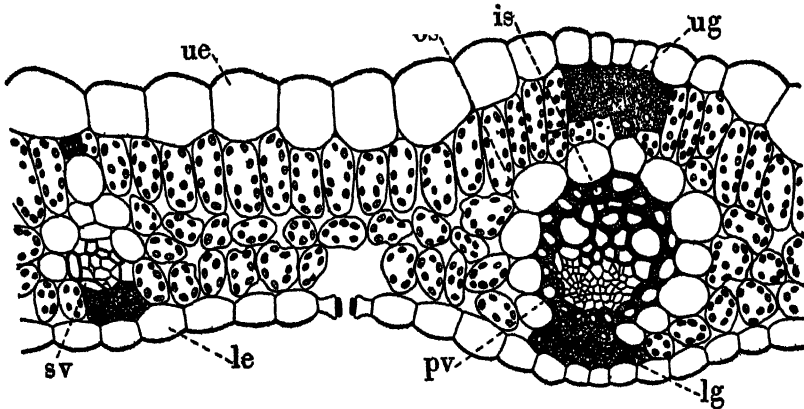


Fig. 4. A portion of a cross-section of the blade to show vascular structure. *ug*, upper girder; *lg*, lower girder; *os*, outer portion of bundle sheath; *is*, inner portion of bundle sheath; *p**v*, primary vein; *s**v*, secondary vein; *ue*, upper epidermis; *le*, lower epidermis.

The midrib and the six large nerves observed in the blade are also present in the sheath. They extend almost parallel from the base upward and converge in the upper region to enter the petiole. Between each pair of large nerves there is only one small vein, but two or three small ones lie between the edges of the sheath and the outermost large nerves (Pl. 24, fig. 1, s). These slender outer veins unite to form a single strand which enters the petiole. Small cross-veins occur in the leaf-sheath, but they are not as numerous here as in the blade.

The longitudinal veins in the sheath are also reinforced by fibrous girders, which are more prominent on the abaxial than on the adaxial side of the bundle. In the central upper portion of the sheath, however, the vascular strands do not come in contact with the girders. The fibers lie just beneath the epidermis while the veins are embedded in the thickened mesophyll.

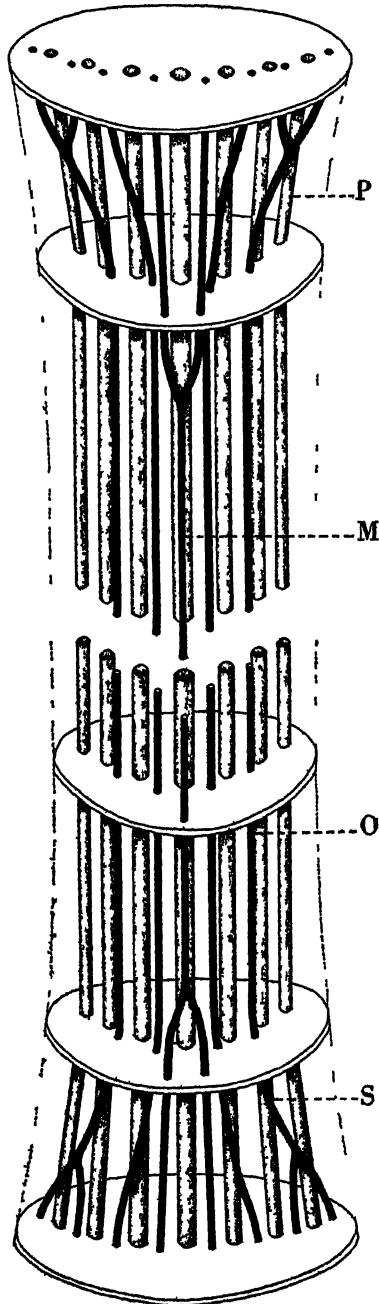


Fig 5. A diagram of the vascular system of the petiole (greatly shortened). *p*, primary vein; *s*, secondary vein, *o*, the single bundle formed by the union of the two outer slender veins, *m*, the single bundle formed by the union of the two middle slender veins.

In order to trace the vascular system through the petiole, several series of slides were prepared from cross-sections of mature leaf-stalks and from young leaves in the bud.

The midrib and the six large nerves observed in both blade and sheath continue without change through the leaf-stalk (fig. 5, p). They lie parallel and close together, forming a crescent in cross-section. Eight of the slender nerves enter the petiole both from the sheath and from the blade (fig. 5, s). In cross-section, these smaller veins alternate with the larger nerves. A short distance from each end of the petiole, the two outermost pairs of small veins unite and extend through the stalk as single strands (fig. 5, o). The six small veins which then remain, lie in a separate group next to the dorsal side of the petiole. In the longer leaf-stalks, the two middle slender veins usually join to form a single bundle (fig. 5, m). Cross-venation does not occur in the petiole.

Fibrous girders do not lie immediately above or below the vascular strands in the leaf-stalk. Some sclerenchyma is present, however, in the ground tissue just beneath the flattened adaxial surface of the middle portion of petiole (Pl. 24, fig. 6). The upper terete end of the stalk does not possess sclerenchyma when young. This absence of strengthening tissue enables the distal part of the petiole to bend and give the leaf a more advantageous position.

ONTOGENY

The best method found for the study of leaf development was to dissect the buds under a binocular microscope and to observe them at high power. All stages in leaf-formation were studied in this way. Further information was gained from both longitudinal and cross-sections of young leaves in the bud.

The leaf primordium arises as a lateral outgrowth from the meristematic tissue at the end of the stem. That part of the primordium which is to become the apex of the leaf appears at first. Further development is basipetal and intercalary. The leaf-primordium expands laterally until it almost surrounds the vegetative point like a ring (Pl. 24, fig. 7).

In the beginning the ridge of embryonal tissue shows neither blade nor sheath. The upper portion of the primordium finally becomes more expanded than the very short basal region, and, as growth continues, the lower portion of the basal segment also expands laterally, leaving a constricted region between the upper and lower parts of the leaf (Pl. 24, fig. 8). The upper and lower expanded parts become the blade and sheath, respectively, and the narrow constricted region forms the leaf-stalk (Pl. 24, fig. 9). This stalk is at first crescent-shaped in cross-section, but later

thickens by an extended period of secondary cell division in its ground tissue.

The procambial strands, which later develop into the longitudinal veins, appear in the leaf primordium. They lie parallel and close together at first, but the subsequent lateral expansion of the blade and sheath spreads them farther apart in those regions. As a result of this intercalary and basipetal leaf development, the venation of the blade becomes arcuate, and the close approximation of the veins in the petiole is not altered.

SUMMARY

1. The foliage leaf of *Zeugites munroana* affords a good example of a petiole inserted between the blade and the sheath.
2. The leaf is convolute in the bud, but there is no definite relationship between the overlapping in the blade and that of the sheath. The blade may be wrapped to the left and its sheath to the right, or vice versa; or both may be coiled with the same edge inward.
3. Seven large primary veins, which maintain their unity, extend through the sheath, petiole, and blade.
4. Many small secondary veins extend through the blade in the spaces between the large primary veins. These secondary strands begin to anastomose as they near the petiole, five or six of them passing through the stalk in a group apart from the larger nerves.
5. The apex of the leaf is the oldest and further growth is basipetal and intercalary.
6. The blade and sheath are differentiated by the lateral expansion of the upper and lower portions of the leaf during development.
7. The petiole is intercalated between the blade and sheath, or comes from that part of the primordium between the two.
8. The longitudinal veins, which appear as procambial strands in the leaf-primordium, lie parallel and close together at first, but the subsequent expansion of the blade and sheath spreads them farther apart in those regions.

The writer wishes to express his appreciation to Dr. Paul Weatherwax, under whose direction the work was carried on, and to whom he is greatly indebted for advice and criticism.

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Explanation of plate 24

Zeugites munroana

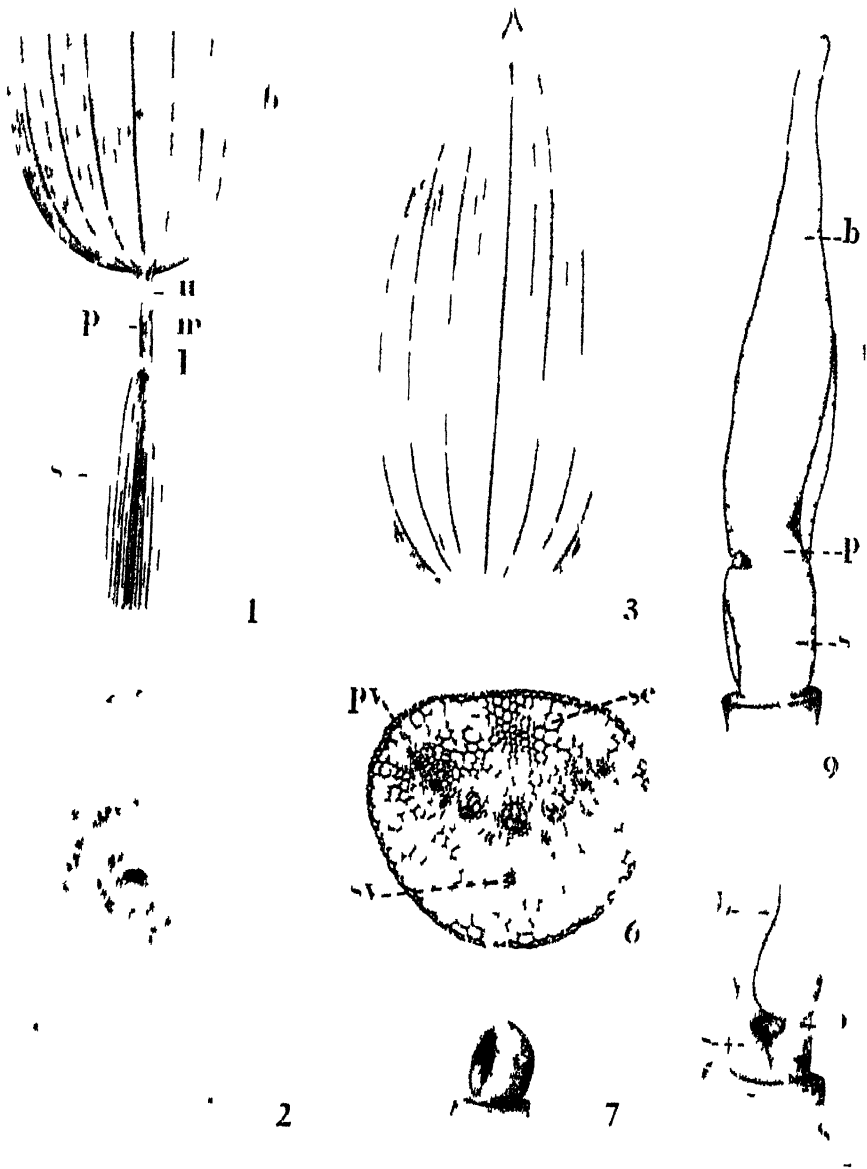
Key to all figures. *b*, blade; *p*, petiole; *u*, upper terete end of petiole; *m*, portion of petiole having flattened adaxial surface; *l*, lower end of petiole which is oval in cross-section; *s*, sheath; *pv*, primary vein; *sv*, secondary vein; *sc*, sclerenchymatous tissue.

Figs. 1, 3. Portions of leaf decolorized, stained, and cleared, showing petiole and venation.

Fig. 2. Cross-section of bud which shows convolute vernation.

Fig. 6. Cross-section through middle portion of young petiole.

Figs. 7-9. Consecutive stages in leaf development.



MCCOY ZEUGITES

Lemanea grandis (Wolle) Atk. rediscovered after forty years

ROBERT B. GORDON¹

In 1877 a species of freshwater red algae new to science was discovered in the vicinity of Bethlehem, Pa. by Rev. Francis Wolle, who described it under two generic names, both wrongly applied. Another collection of the same species was made in the neighboring state of Delaware about ten years later. For over forty years there has been no published record of its occurrence elsewhere. Not that it is one of those microscopic plants which can be easily overlooked, because it grows in olive-brown tufts or clusters of thalli an inch or more in length, attached to rocks in the clear waters of mountain brooks and rivers.

It is with much satisfaction that we can announce another locality for this apparent rarity of the plant kingdom, in Western New York State, where the Allegheny River makes a sharp bend in its course, south of the village of Quaker Bridge. It has also been found seven or eight miles to the east on a small tributary known as Quaker Run, in the Allegany State Park (Mathews, 1932).

Species of Rhodophyceae or "red algae" in fresh water are of such infrequent occurrence that any new locality record should be worthy of note. According to G. M. Smith (1933) there are only six freshwater genera of the larger sub-class Florideae, comprising some twenty-three species, found in the United States. Of these, ten species belong to the genus *Batrachospermum* and eight are species of *Lemanea*, seven of which are known from two or more stations.

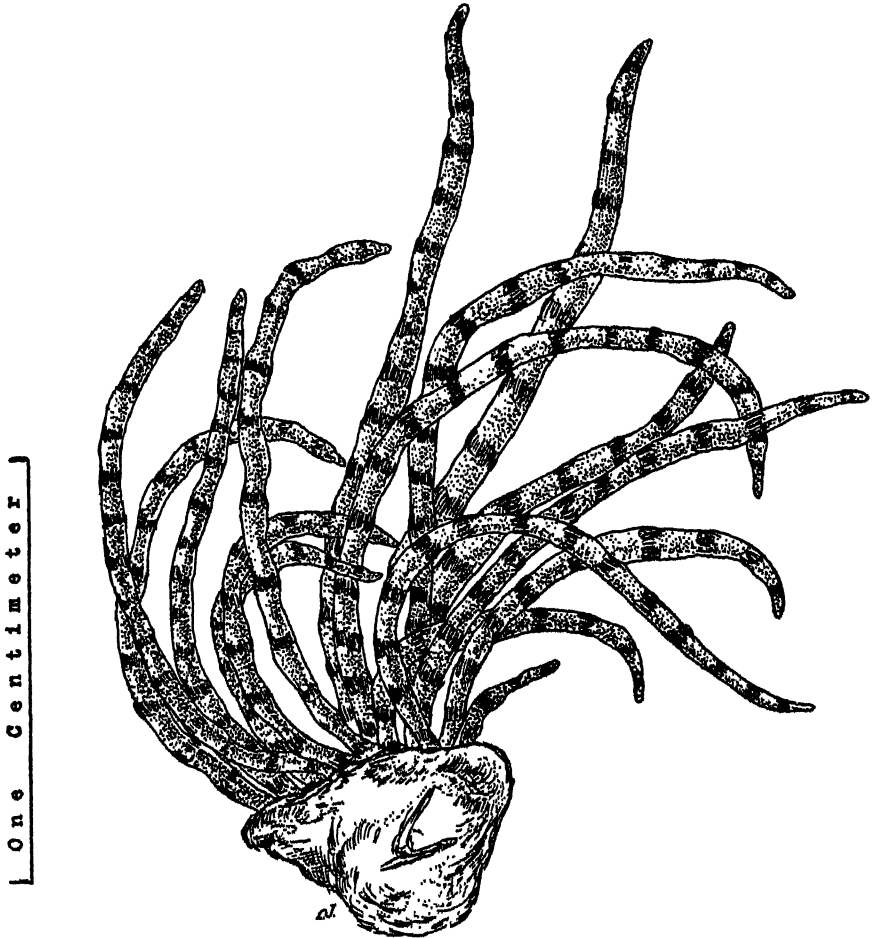
At the time of publication of Wolle's "Freshwater Algae of the United States" in 1887, *Lemanea grandis* was certainly known from only two stations, and there have been no collections since, except that a record of *Tuomeya* by Neebham and Strausbaugh (1930) may possibly refer to this species. The two stations were in shallow, sluggish, river water, Bethlehem, Pa., by Wolle in 1877, and at Falkland, Del., by A. Commons in 1886 (Atkinson, 1931).

Wolle described the specimens which he collected as a new species of *Tuomeya*, a genus made by Harvey in 1858. G. F. Atkinson (1890) furnished the following important notes about Wolle's original material: "I have examined specimens of this species from Wolle's herbarium and from Rabenhorst's Alg. Europ, No. 2538, and find it to be a *Lemanea*. Through the kindness of Dr. W. G. Farlow I have had the opportunity of examining

¹ Papers from the Department of Botany, the Ohio State University, No. 342.

specimens of *Tuomeya fluviatilis* Harv., from Harvey's Herbarium. It is very different from Wolle's *Tuomeya grande* (*Entothrix grande* Wolle). I have made careful dissections and find Wolle's *Entothrix* is identical with characters of the subgenus *Lemanea*."

Lemanea and *Tuomeya* . . . may be distinguished from each other by



Lemanea grandis (Wolle) Atkinson. Habit sketch by Louis Jacobson.

the differentiation into nodes and internodes in *Lemanea* and the lack of them in *Tuomeya* (Smith, 1933). The subgenus *Eulemanea* to which *L. grandis* belongs is recognizable by antheridial zones that encircle the thallus in an even transverse band or belt at the nodes (see figure). The only other species of *Lemanea* found in New York State is *L. fucina* Bory, in which the antheridial zones are more or less warty, and the antheridia are con-

finned to these protuberances, which are not usually in lateral contact with one another, an external characteristic of the subgenus *Sacheria* (Atkinson, 1931).

The drawing which accompanies this article shows the appearance of a tuft of thalli in the summer condition. The specimen from which the drawing was made was collected in the Allegheny River below Quaker Bridge, N. Y., which is located in the Alleghany Indian Reservation. Dr. Robert E. Coker, Director of the Alleghany School of Natural History, called my attention to this plant in 1930, having discovered it the previous summer. L. A. Kenoyer, the botanist, noted "the occurrence in Quaker Run near the school and in places along the Allegheny River of *Tuomeya grande* (or a closely related species)" in his report to the Director in 1929.

Duplicate material from the latter locality has been sent to Prof. Dr. H. Skuja, of the Botanical Institute at the University of Riga, Latvia. Dr. Skuja made the following comment: ". . . you have determined correctly the *Lemanea* as *L. grandis* (Wolle) Atkinson. Indeed this *Eulemanea* is characterized very well by its low stout box-shape and its very large roundish carpospores. Otherwise it resembles closely *L. australis* Atkins., which however contains more elongated and somewhat smaller carpospores." (Translation from letter dated February 20, 1934.)

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Scrophulariaceae of the Northwestern United States—II.

Pedicularis of the Group Bracteosae

FRANCIS W. PENNELL

The group of *Pedicularis bracteosa*, which as a section of that genus may be called the Bracteosae, occurs wholly in the northwestern United States. Its members are closely similar in habit, being erect, fleshy-stemmed plants with leaves deeply once pinnatifid and so wide as to be often equilaterally triangular in their general outline. In strong contrast to the leaves, the bracts are short, wide, and nearly or quite entire. The calyx-lobes are five, and prominently developed. The corolla, either yellow or purple or combining both colors, has the galea raised somewhat above the anterior lip and terminating bluntly or in a sharp tip or slight beak. The capsules are short, with both cells relatively well developed. The seeds are reticulate, or longitudinally ridged or slightly winged.

It is not surprising that plants so alike in aspect and nearly associated in range should have long passed as a single species. *Pedicularis bracteosa* was established by Bentham in 1838 for a plant collected by both Drummond and Douglas in the Canadian Rocky Mountains. In 1886 Gray recognized the distinctness of *P. canbyi* from the Mission Range of northwestern Montana, and in 1907 Rydberg described *P. siifolia*, also from western Montana and the only other species with beaked galea. Only one segregate has ever been proposed from the remainder of the group, although there exist outstanding differences in the degree of union of and the occurrence of glandularity on the calyx-lobes, the character of the pubescence, and the color of the corolla; curiously enough, *P. montanensis* Rydb., described from Montana in 1897, proves to be identical with typical *bracteosa*. Analysis shows that among these plants with beakless galea, there are about seven species awaiting scientific recognition.

Although it has been several years since my first detection of these entities, their description has been withheld until after the expedition of 1931 into the northwestern states, when nearly all of these species were seen in flower. Next, it was my intention to embody the account of them in a larger study of "The American Species of *Pedicularis*," but the necessity of completing in the near future other tasks has caused such delay that it appears advisable to present now this limited treatise of the immediate group of *Pedicularis bracteosa*.

Specimens have been seen from many herbaria, to the curators of which I am indebted. In a later paper these herbaria will be enumerated, and specimens cited so as to portray accurately the range of each species. At present I will merely acknowledge my special obligations to the New York

Botanical Garden, where are the types of the species described by the late Dr. Per Axel Rydberg; to the United States National Herbarium, ever the most representative collection of the United States and which contains the herbarium of Charles V. Piper; to the Rocky Mountain Herbarium of the University of Wyoming, under the care and stimulus of Professor Aven Nelson, and where the late Dr. Edwin B. Payson studied; and to the State College of Washington, where Dr. Piper long worked and where until recently Dr. Harold St. John was continuing his labors. I have especially appreciated the critical assistance of Dr. St. John.

Opportunities for field study were made possible in 1915 by the New York Botanical Garden, and in 1931 by the Academy of Natural Sciences, aided by a grant from the National Research Council. On the earlier trip to the central Rocky Mountain states *Pedicularis paysoniana* was repeatedly seen. While on the latter journey through the northwestern states nearly all the remaining species of the group were collected and studied.

KEY TO SPECIES

Galea abruptly strongly deflexed near apex, beakless.

Lobes of calyx distally filiform, glandular-pubescent, the free lateral lobes longer than their united basal portions.

Corolla dark red throughout, 20 mm. long; glands of calyx-lobes large, blackish; bracts 15–20 mm. long, lanceolate with caudate tip, much exceeding the calyx.

1. *P. atrosanguinea*

Corolla purple or partly yellow; glands of calyx-lobes small, pale; bracts shorter.

Corolla 13–16 mm. long, the anterior lip ciliolate; capsule 2–3 mm. long, light brown; bracts oval, with caudate tips.

2. *P. bracteosa*

Corolla 18–20 mm. long, the anterior lip not ciliolate; capsule 9–11 mm. long, dark brown; bracts lanceolate-caudate.

3. *P. paddoensis*

Lobes of calyx lanceolate to linear, not or scarcely glandular.

Calyx-lobes linear, the free lateral lobes longer than their united basal portions.

Inflorescence glabrous or nearly so; free lateral calyx-lobes less than twice as long as the united basal portions; corolla greenish-yellow.

4. *P. flavida*

Inflorescence villose (except at times in *P. Thompsonii*); calyx-lobes usually more than twice as long as their united basal portions.

Galea in anthesis raised little above the anterior lip, and usually enfolded by it; leaf-blades with 6 or 8 pairs of wider dentate segments; roots tuberous-thickened; corolla greenish-yellow.

5. *P. pachyrhiza*

Galea in anthesis raised well above the anterior lip; leaf-blades with 8 to 12 pairs of narrow doubly and sharply toothed segments.

Corolla pale yellow; bracts (except the lowermost) lanceolate-caudate; roots not tuberous-thickened.

6. *P. Paysoniana*

Corolla with galea purple and the anterior lip yellow; bracts (except the

lowermost) ovate, rather abruptly narrowed to a more slender caudate tip; roots slightly tuberous-thickened.

7. *P. Thompsonii*

Calyx-lobes triangular-lanceolate, the free lateral lobes shorter than their united basal portions; corolla either wholly yellow or with purple galea.

8. *P. latifolia*

Galea less sharply decurved, cuneately narrowed to an acute, slightly beaked apex; calyx-lobes united much higher laterally than medianly; corolla greenish-yellow.

Anterior lobes of corolla oval, entire or nearly so; bracts with caudate tip about as long as the oval body; inflorescence villose to glabrate; stem 3-7 dm. tall, the roots tuberous-thickened.

9. *P. siifolia*

Anterior lobes of corolla flabellate, erose-denticulate; bracts with caudate tip shorter than the widely ovate body; inflorescence villose; stem 1-1.5 dm. tall, the roots not tuberous-thickened.

10. *P. Canbyi*

1. *Pedicularis atrosanguinea* Pennell & Thompson, sp. nov.

Roots slightly tuberous-thickened. Stem 3-7 dm. tall, glabrous below the inflorescence. Leaves alternate, mostly cauline, the basal on long petioles, the cauline nearly sessile, the blades 6-13 cm. long, over 2/3 as wide, bipinnatifid, divided nearly or quite to the midrib into 8 to 12 pairs of segments which are nearly regularly serrate or dentate. Inflorescence nearly spicate, densely flowered, 9-15 cm. long, the rachis, bracts and calyces moderately lanose with glandless hairs, the calyx-lobes pubescent with black-headed glands. Bracts 15-20 mm. long, lanceolate-caudate, entire or distally slightly serrate. Pedicels less than 2 mm. long. Calyx 9-12 mm. long, the posterior sepal about 2/3 the length of the others, as a free lobe projecting 2-3 mm. from the posterior cleft of the calyx-tube, the lateral sepals united slightly higher, even, free 5-6 mm., linear-filiform, attenuate, lobes all entire. Corolla about 20 mm. long, dark blood-red (drying nearly black); the galea erect, distally decurved, at apex abruptly truncate, without teeth; the anterior lip ascending but so much shorter than galea as not to close the throat, the anterior lobes somewhat erose. Filaments and anthers glabrous. Capsule 12 mm. long, flattened, 7 mm. wide, with decurved tip, the cells slightly unequal, dehiscing to base on upper side and opening anterior cell by a split of the septum. Seeds 3 mm. long, with several low transversely lined wings.

(Caulis 3-7 dm. altus, glaber; folia alterna, 6-13 cm. longa, 4-10 cm. lata, segmentis serratis dentatisve insecta; inflorescentia pilis glanduliferis nigris oblecta; calycis segmenta 5 lineari-filiformia; corolla 20 mm. longa, atrosanguinea, galea crostrata; capsula 12 mm. longa; semina 3 mm. longa.)

Type, openings in coniferous forest, slope of second peak of Mount Angeles, Clallam County, Washington, altitude 1500-1600 meters (5000-6000 feet), collected in flower and fruit August 9, 1931 by F. W. Pennell and J. W. Thompson, no. 15822; in Herb. Academy of Natural Sciences of Philadelphia.

Moist wooded slopes and open meadows, Olympic Mountains, northwestern Washington.

This is a handsome plant, with flowers remarkable for the peculiar color to which the name "atrosanguinea," meaning "dark blood," is especially appropriate.

2. *PEDICULARIS BRACTEOSA* Benth.

Pedicularis bracteosa Benth.; Hook., Fl. Bor. Amer. 2: 110. 1838. "Shady alpine woods of the Rocky Mountains. Drummond. N. W. Am. Douglas (last journey)." Type, collected by Drummond in "Saskatchewan" (by which would surely be meant the Rocky Mountains of the present Alberta), seen in Herb. Kew Gardens. It shows well the lanceolate-attenuate, glandular-pubescent calyx-lobes of the plant considered.

Pedicularis montanensis Rydb. in Bull. Torrey Bot. Club 24: 292. 1897. "Type: J. H. Flodman, no. 796, from Little Belt Mountains, nine miles from Barker [Montana], August 18, 1896." Type seen in Herb. New York Botanical Garden; isotype in Herb. Academy of Natural Sciences of Philadelphia. Rydberg identified *P. paysoniana* as true *bracteosa*, and described again Bentham's original plant under the present name.

Shaded woodland and cool grassy slopes, mountains, Rocky Mountains and contiguous chains, Alberta and British Columbia to western Montana and the Wallowa Mountains of northeastern Oregon.

3. *Pedicularis padoensis* Pennell, sp. nov.

Roots slightly tuberous-thickened, 6 mm. in diameter. Stem 3-6 dm. tall, glabrous below the inflorescence. Leaves alternate, mostly cauline, the basal with moderately long petioles, the cauline nearly sessile, the blades 10-12 cm. long, nearly as wide bi- or tri-pinnatifid, divided nearly or quite to the midrib into 10-12 pairs of segments, which are doubly serrate-dentate. Inflorescence essentially spicate, densely flowered, 8-15 cm. long, the rachis, bracts and calyces glandular-puberulent or slightly hairy. Bracts 15-20 mm. long, lanceolate, caudate, distally slightly serrate. Pedicels less than 1 mm. long. Calyx 10-12 mm. long, the posterior sepal slightly the shortest, as a free lobe projecting 4-6 mm. from the deep posterior cleft of the calyx-tube, the lateral sepals nearly even, free 4-7 mm. from apex, lobes all entire. Corolla 18-20 mm. long, purple or sometimes at least the lip yellow; the galea erect, distally decurved, at apex truncate, without teeth; the anterior lip ascending, shorter than the galea and leaving the throat open, the anterior lobes merely crose. Filaments and anthers glabrous. Capsule 9-10 mm. long, dark brown. Seeds 3 mm. long, the pale testa slightly ridged, and prominently cross-lined.

(Caulis 3-6 dm. altus, glaber; folia alterna, 10-12 cm. longa, 9-11 cm. lata, segmentis duplicate serrato-dentatis insecta; inflorescentia glandulopuberulentia; calycis segmenta 5 lineari-filiformia; corolla 18-20 mm. longa, tota purpurea vel labio anteriore flava, galea erecta; capsula 9-10 mm. longa; semina 3 mm. longa.)

Type, moist rich soil in coniferous woodland, bank of Bird Creek, altitude 1700-1800 meters (5500-6000 feet), Mount Adams, Yakima County, Washington, collected in late flower and fruit July 31, 1931, by F. W. Pennell, no. 15738; in Herb. Academy of Natural Sciences of Philadelphia.

Moist woods and meadows, upper slopes of Mount Adams (Mount Paddo), Cascade Range, Washington.

4. *Pedicularis flavida* Pennell, sp. nov.

Roots tuberously thickened. Stem 3–9 dm. tall, glabrous throughout. Leaves alternate, mostly cauline, the basal with long petioles, the cauline nearly sessile, the blades 5–10 cm. long, nearly as wide, bipinnatifid, divided nearly or quite to the midrib into 10 to 14 pairs of segments, which are irregularly serrate or with again serrate lobes. Inflorescence nearly spicate, rather loosely flowered, 7–15 cm. long, the rachis, bracts and calyces glabrous or sparsely puberulent. Bracts 10–20 mm. long, ovate-caudate to lanceolate, entire or distally slightly serrate. Pedicels less than 1 mm. long. Calyx 8–10 mm. long, the posterior sepal slightly the shortest, as a free lobe projecting 2–3 mm. from the deep posterior cleft of the calyx-tube, the lateral sepals even, free 3–4 mm. from apex, lobes all entire. Corolla 15–18 (–20) mm. long, greenish yellow (pale viridine yellow), or rarely the anterior lip (at base) purple; the galea erect, distally decurved, at apex truncate, without teeth; the anterior lip ascending, shorter than the galea, nearly closing the orifice to the throat, the anterior lobes cross. Filaments and anthers glabrous. Capsule 8–10 mm. long, flattened, 4–5 mm. wide, with decurved tip, the cells slightly unequal, capsule dehiscent to base on posterior side and then splitting septum. Seeds 2.5 mm. long, with several low, transversely lined ridges.

(Caulis 3–9 dm. altus, glaber; folia alterna, 5–10 cm. longa, 4–9 cm. lata, segmentis serratis biserratisve insecta; inflorescentia glabra; calycis segmenta lineari-filiformia; corolla 15–18 mm. longa, ochroleuca, galea erecta; capsula 8–10 mm. longa; semina 2.5 mm. longa.)

Type, wet thicket along Elk Lake, Deschutes County, Oregon, altitude 1430–1550 meters (4700–5100 feet), collected in flower July 9, 1931, by F. W. Pennell, no. 15545; in Herb. Academy of Natural Sciences of Philadelphia.

Moist slopes, meadows and open woods, high mountains, Cascade Range of Oregon and Siskiyou Mountains of southern Oregon and northern California.

5. *Pedicularis pachyrhiza* Pennell, sp. nov.

Roots tuberous-thickened, the larger 6–8 mm. in diameter. Stem 4–10 dm. tall, glabrous and slightly glaucous below the inflorescence. Leaves alternate, mostly cauline, the basal on long petioles, the cauline nearly sessile, the blades 10–16 cm. long, 2/3–3/4 as wide, bipinnatifid, divided to the midrib into 6 or 8 pairs of segments, which are doubly crenate-dentate. Inflorescence essentially spicate, densely flowered, 10–15 cm. long, the rachis, bracts and calyces lanose. Bracts 13–25 mm. long, lanceolate-caudate, distally crenate-serrate. Pedicels less than 1 mm. long. Calyx 15 mm. long, the posterior sepal less than 1/2 the length of the others, as a free lobe projecting 1–4 mm. from the

very deep posterior cleft of the calyx-tube, at times hardly evident, the lateral sepals unequal, hardly united higher laterally, antero-lateral pair slightly shorter, postero-lateral pair free 4–9 mm. from apex, lobes all entire. Corolla 15–22 mm. long, greenish yellow; the galea erect, chalcedony yellow, distally decurved at apex, truncate, without teeth; the anterior lip ascending, light green yellow, nearly as long as the galea but hardly closing the throat, the anterior lobes entire and not ciliolate. Filaments and anthers glabrous. Capsule 10–11 mm. long, with decurved tip, the cells slightly unequal, dehiscing to base on posterior side and opening anterior cell by a longitudinal split of the septum (later also dehiscing on anterior side). Seeds not seen.

(Caulis 4–10 dm. altus, glaber; folia alterna, 10–16 cm. longa, 7–12 cm. lata, segmentis duplicate crenato-dentatis insecta; inflorescentia lanosa; calycis segmenta linearia; corolla 15–22 mm. longa, ochroleuca, galea erostrata; capsula 10–11 mm. longa; semina non visa.)

Type, bushy summit, altitude 1500 meters (5000 feet), Blue Mountains northwest of Elgin, Union County, Oregon, collected in flower July 2, 1931 by F. W. Pennell, no. 15414; in Herb. Academy of Natural Sciences of Philadelphia.

Open glades in woods, Blue Mountains of northeastern Oregon.

6. *Pedicularis Paysoniana* Pennell, sp. nov.

Roots not tuberous-thickened. Stem 3–9 dm. tall, glabrous below the inflorescence. Leaves alternate, mostly cauline, the basal on long petioles, the cauline shortly petioled, the blades 5–15 cm. long, from 2/3 to nearly as wide as long, bi- or usually tri-pinnatifid, divided to the midrib into 9 to 12 pairs of segments which are irregularly toothed or cut into serrate lobes. Inflorescence essentially spicate, densely flowered, 10–30 cm. long, the rachis, bracts and calyces loosely lanate. Bracts 10–20 cm. long, lanceolate, slightly caudate, distally slightly serrate. Pedicels less than 1 mm. long. Calyx 8–10 mm. long, the posterior sepal about 2/3 the length of the others, as a free lobe projecting 2 mm. from the posterior cleft of the calyx-tube, the lateral sepals united little higher (occasionally even half their free length), nearly even, free 4 mm. from apex, lobes all entire. Corolla 20 mm. long, yellow; the galea erect, distally decurved, at apex abruptly truncate and without teeth; the anterior lip ascending but so much shorter than the galea as not to close the throat, the anterior lobes slightly erose, not ciliate. Filaments and anthers glabrous. Capsule 10–11 mm. long, with mucronate decurved tip, the cells slightly unequal, dehiscing to base on posterior side and opening anterior cell by a longitudinal split of the septum. Seeds 4 mm. long, obovoid, reticulate especially with prominent transverse lines.

(Caulis 3–9 dm. altus, glaber; folia alterna, 5–15 cm. longa, 4–14 cm. lata, segmentis dentato-serratis lobatisve insecta; inflorescentia lanosa; calycis segmenta linearia; corolla 20 mm. longa, flava, galea erostrata; capsula 10–11 mm. longa; semina 4 mm. longa.)

Type, moist slope, mountains near Cottonwood Lake, east of Smoot, Lincoln County, Wyoming, collected in flower August 2, 1923, by E. B. Payson and G. M. Armstrong, no. 3724; in Herb. Academy of Natural Sciences of Philadelphia.

Moist woodland, at altitudes of 2200 to 3600 meters, mountains of the Rocky Mountain system, central Montana southward through Wyoming and eastern Idaho to Colorado and the La Sal Mountains of southeastern Utah.

It is a pleasure to associate with this beautiful and characteristic species of the central Rocky Mountains the names of Dr. and Mrs. Edwin Blake Payson. The untimely death of Dr. Payson in 1927 removed from American taxonomy not only one of its most promising students but the one whose work seemed most likely to aid in the understanding of the Rocky Mountain flora.

7. *Pedicularis Thompsonii* Pennell, sp. nov.

Roots slightly tuberous-thickened. Stem 3-8 dm. tall, glabrous below the inflorescence. Leaves alternate, the basal on long petioles, the cauline shortly petioled or sessile, the blades 8-10 cm. long, from $1/2$ - $2/3$ as wide, bipinnatifid, divided to the midrib into 8 to 10 pairs of segments which are sharply toothed and the teeth crenate-serrate. Inflorescence spicate, densely flowered, 10-15 cm. long, the rachis, bracts and calyces loosely lanose or glabrescent (with the latter ciliate). Bracts 10-15 mm. long, ovate, with a narrow caudate, serrate or entire tip. Pedicels about 1 mm. long. Calyx 8-11 mm. long, the posterior sepal $3/5$ - $2/3$ the length of the others, as a free lobe projecting 3-5 mm. from the posterior cleft of the calyx-tube, the lateral sepals united farther, nearly even, free 3-7 mm. from apex, lobes all entire. Corolla 16-19 mm. long, purple and partly yellow; the purple galea erect, distally decurved, at the yellow apex abruptly truncate, without teeth; the anterior lip yellow, ascending but so much shorter than the galea as not to close the throat, the anterior lobes slightly erose and at times ciliolate. Filaments and anthers glabrous. Capsule 8-10 mm. long, with decurved tip, the cells slightly unequal, dehiscing to base on posterior side and opening anterior cells mainly by a longitudinal split of septum. Seeds not seen.

(Caulis 3-8 dm. altus, glaber; folia alterna, 8-10 cm. longa, 4-7 cm. lata, segmentis duplicate serrato-dentatis insecta; inflorescentia lanosa glabrescensve; calycis segmenta linearia; corolla 16-19 mm. longa, purpurea vel parte flava, galea erecta; capsula 8-10 mm. longa; semina non visa.)

Type, open woods near Wauconda, summit between Tonasket and Republic, Okanogan County, Washington, collected in flower June 29, 1931, by J. William Thompson, no. 7142; in Herb. Academy of Natural Sciences of Philadelphia.

Mountains of northern Washington.

Of uncertain status, and possibly hybrid origin.

8. *Pedicularis latifolia* Pennell, sp. nov.

Roots tuberous-thickened. Stem 4–12 dm. tall, glabrous below the inflorescence. Leaves alternate, mostly cauline, the basal with long petioles, the cauline nearly sessile, the blades 5–20 cm. long, often nearly as wide, paler beneath, bi- or tri-pinnatifid divided nearly to the midrib into 8 to 12 pairs of segments, which are irregularly serrate, toothed or with serrate lobes. Inflorescence nearly spicate, densely flowered, 10–40 cm. long, the rachis, bracts and calyces usually slightly pubescent or slightly lanose. Bracts 11–20 mm. long, lanceolate to ovate, caudate, entire or rarely serrate. Pedicels less than 1 mm. long. Calyx 10–12 mm. long, the posterior sepal much shorter than the others, as a free lobe projecting 3–5 mm. from the deep posterior cleft of the calyx-tube, the lateral sepals even, those of each side united to within 2–4 mm. of apex, but anteriorly united only a little farther than posteriorly, the lobes entire. Corolla 15–18 (–20) mm. long, yellow (often the bend or the entire galea purple); the galea erect, distally decurved, at apex abruptly truncate, without teeth; the anterior lip ascending but so much shorter than the galea as not to close the throat, the anterior lobes entire or slightly erose. Filaments glabrous throughout; anthers glabrous. Capsule 9–10 mm. long, with mucronate decurved tip, the cells unequal, dehiscing to base on posterior side and opening anterior cell by a longitudinal split of the septum. Seeds 3 mm. long, ovoid, finely ridged, with reticulate testa, the longitudinal ridges of which are drawn out in several low, thin wings.

(Caulis 4–12 dm. altus, glaber; folia alterna, 5–20 cm. longa, 3–18 cm. lata, segmentis duplicate dentatis serratisve insecta; inflorescentia pubescentia; calycis segmenta lateralia fere juncta; corolla 15–18 mm. longa, flava vel galea purpurea, galea erecta; capsula 9–10 mm. longa; semina 3 mm. longa.)

Type, openings in coniferous forest, Paradise Inn, Mount Ranier, Washington, altitude 1600–1800 meters (5200–6000 feet), collected in flower and fruit August 5–6, 1931, by F. W. Pennell, no. 15786; in Herb. Academy of Natural Sciences of Philadelphia.

Moist open woods and grassy slopes, high mountains of southern British Columbia, Washington and Northern Idaho, from the Cascade Mountains to the Cœur d'Alene Mountains.

9. *PEDICULARIS SIIFOLIA* Rydberg

Pedicularis siifolia Rydb in Bull. Torrey Bot. Club 34: 35. 1907. "Montana: Grant Creek, June 7, 1897, M. J. Elrod, and assistants 97." Type seen in Herb. New York Botanical Garden.

Moist dark woods, mountains of central Idaho, northward to Missoula County, Montana, and westward to Asotin County, Washington.

10. *PEDICULARIS CANBYI* Gray

Pedicularis Canbyi Gray, Syn. Fl. N. Amer. 2. I: 454. 1886. "Rocky Mountains of Montana, on McDonald's Peak of the Mission Range, at 8400 feet, Canby, 1883." Isotype, W. M. Canby 266, collected July 19, 1883, seen in Herb. Academy of Natural Sciences of Philadelphia.

Mission Range of northwestern Montana.

ACADEMY OF NATURAL SCIENCES OF PHILADELPHIA

Descriptions of ten new hybrid poplars

E. J. SCHREINER and A. B. STOUT

The ten hybrid poplars here described have been selected as the most promising plants among 69 hybrids which in turn were chosen from a total of approximately 13,000 individual seedlings obtained by hybridizing 34 different types of poplars. Summaries of this breeding project have already been published¹ which record the methods employed, the names of the poplars used as parents, the number of seedlings grown in each hybridization, the basis upon which selections were made, and the particular crosses which yielded selection plants. This paper will be devoted to the ten hybrids which have thus far appeared to be best in respect to (a) vigor of growth in the nursery of the Oxford Paper Company at Frye, Maine, (b) ability to root from cuttings, (c) hardiness, and (d) resistance to disease.

The descriptions are of necessity based on nursery stock and young trees, and hence the sex, the form of the tree, and the character of the trunk can not be given until later when mature trees are seen. It is well known that, for poplars especially, there are decided differences in the appearance of the leaves on vigorous nursery stock and on older trees. Also there are noticeable contrasts in the size and shape of leaves on vigorous end-shoots compared with those of slow-growing, lower, and lateral branches. Also differences in size and shape exist for a series of leaves on the same branch. The following descriptions include consideration of both primary and secondary branches and of the character of the twigs and buds in summer and in winter.

Each of these ten hybrids is being propagated by cuttings to obtain numerous trees all of which are merely branches of the original seedlings with the same individual nature and status. To each of these clones the authors have applied a special horticultural name which has some association with the hybridization project.

GENEVA POPLAR

Populus Maximowiczii (♀) × *P. berolinensis* (♂)

SUMMER CHARACTERS. *Stems.* Round throughout, somewhat sparsely pubescent except at base of most vigorous shoots, reddish brown toward

¹ The breeding of forest trees for pulpwood. A. B. Stout, Ralph H. McKee, E. J. Schreiner. Jour. New York Bot. Garden 28: 49-63. 1927.

Results of a project in hybridizing poplars. A. B. Stout and E. J. Schreiner. Jour. of Heredity 24: 216-229. 1933.

tip, olive green toward base; lenticels white and linear toward tip, pinkish brown and broadly linear or oval toward base, 0.5–3.0 mm. long. *Leaves*. Broadly ovate to oval, apex acute to short-pointed, base obtuse; firm, leathery, rather dark dull green above, glaucous below; margin medium finely crenate, usually 4–5 crenations per cm., glandular, quite strongly undulate; midrib and veins green and sparsely pubescent above (in very young leaves tinted red), green and glabrous below. Petiole round, reddish and sparsely pubescent on upper side, green and glabrous below, lenticellate, 32–44 mm. long. Stipules rather narrow subulate, long pointed, rather early fugaceous. Leaves on secondary shoots, elliptical, apex acute to short pointed, base acute; margin more finely crenate, usually 5–7 crenations per cm. *Buds*. Rather broadly lanceolate, sharp-pointed, 6–10 mm. long, glossy dark reddish brown, viscid, aromatic, appressed.

WINTER CHARACTERS. *Stems*. Round, slightly ridged, slightly pubescent, and greyish brown toward tip, greyish green toward base; lenticels reddish pink, linear toward tip, elliptical to circular toward base; pith somewhat five-sided toward tip, round toward base, light brown, homogeneous. Leaf-scars broadly triangular, decurrent, winged and keeled by short rounded ridges; three prominent bundle-scars. Stipule-scars rather inconspicuous, linear, somewhat curved. *Buds*. Terminal buds ovate. Axillary buds narrowly ovate with acute tips, light brown to dark reddish brown, 8–12×4–6 mm., resinous, viscid, aromatic, appressed.

OXFORD POPLAR

P. Maximowiczii (♀) × *P. berolinensis* (♂)

SUMMER CHARACTERS. *Stems*. Round, somewhat sparsely pubescent, brownish red toward tip, olive green toward base; lenticels white and linear toward tip, pinkish brown and oval toward base, 0.5–1.0 mm. long. Secondary shoots somewhat less red. *Leaves*. Broadly ovate to oval, apex short acute, base obtuse, very slightly cordate at junction of blade and petiole; firm, rather leathery, dull dark green above, glaucous below; margin medium finely crenate, usually 3–5 crenations per cm., glandular, strongly undulate; midrib and basal portion of large veins red and puberulose above, (in older leaves red color restricted to basal portion of midrib) green and glabrous below. Petioles round to broadly oval, red and puberulose above, green and sparsely puberulose below, lenticellate, 30–50 mm. long. Stipules rather small, almost triangular, green, somewhat tardily fugaceous. Leaves on secondary shoots broadly elliptical, apex short acute, base broadly acute to obtuse; margin more finely crenate, usually 5–7 crenations per cm. *Buds*. Lanceolate, 6–10 mm. long, somewhat glossy, red to reddish brown; viscid, aromatic, appressed.

WINTER CHARACTERS. *Stems.* Round, reddish brown and sparsely pubescent toward tip, olive grey or greenish toward base; lenticels pinkish, linear toward tip, elliptical to oval or circular toward base; pith five-sided, light brown and homogeneous. Leaf-scars broadly triangular, decurrent, winged and keeled by short, prominent, rounded ridges; three prominent bundle-scars. Stipule-scars often rather indistinct, linear to narrow V-shaped. *Buds.* Terminal buds ovate; axillary buds broadly lanceolate with narrowly acute tips, 10–14×3–5 mm., reddish brown; resinous, viscid, aromatic, closely appressed.

The GENEVA POPLAR and the OXFORD POPLAR are sister seedlings. The seed parent is *Populus Maximowiczii*, a species of the balsam group, which matures fruit in autumn. The pollen parent, although known under the species name *P. berolinensis*, is usually considered to be a hybrid, but its characters are strongly balsam. These two hybrids are to be classed as balsam poplars; the buds are very resinous and the under surface of the leaves is glaucous white. They resemble, rather strongly, the female parent but they root more readily from cuttings, are more hardy, the leaves on the main shoots are conspicuously less orbicular and more acute, and the leaves on the spur shoots are more elliptical and cuneate. The two differ from each other by slight but rather constant features; for the OXFORD POPLAR, the midrib and veins of the older leaves are more red, the pubescence on the midrib and base of the larger veins is shorter, the petioles are longer, and the winter buds are less broad.

ANDROSCOGGIN POPLAR

P. Maximowiczii (♀) × *P. trichocarpa* (♂)

SUMMER CHARACTERS. *Stems.* Round, slightly ridged toward tip, sparsely pubescent except at base of most vigorous shoots, reddish green with occasional green areas toward tip, olive green toward base; lenticels white and linear toward tip, pinkish-brown and broadly linear or oval toward base, 0.5–3.0 mm. long. Secondary shoots round, not ridged toward tip, somewhat more red. *Leaves.* Elliptical to ovate, apex acute, base obtuse to very slightly cordate in largest leaves; firm, leathery, dull dark green above, glaucous below; margin somewhat finely crenate, usually 4–6 crenations per cm., glandular, quite strongly undulate. Midrib and larger veins reddish and sparsely pubescent above (in oldest leaves practically green), green and more sparsely pubescent below. Petioles round, red above, green below, lenticellate, rather sparsely pubescent on both upper and under side, 25–38 mm. long. Stipules subulate, sharp pointed, green, fugaceous. Leaves on secondary shoots elliptical, apex acute, base acute

to obtuse; margin more finely crenate, usually 6–9 crenations per cm.; veins and petioles less red. *Buds*. Lanceolate, sharp pointed, 8–13 mm. long, glossy, dark reddish brown, viscid, aromatic, appressed.

WINTER CHARACTERS. *Stems*. Round with narrow corky ridges on upper portion, finely pubescent and reddish brown toward tip, olive green toward base; lenticels pinkish-brown, linear toward tip, elliptical or oval toward base; pith five-sided near tip, almost circular toward base, light brown, homogeneous. Leaf-scars prominent, broadly triangular, decurrent, winged and keeled by sharp narrow corky ridges, these ridges extending to the next lower bud except at base of stem; three prominent bundle-scars. Stipule-scars rather prominent, linear to narrow V-shaped and slightly curved, quite long. *Buds*. Terminal buds ovate to oval; axillary buds broadly lanceolate with acute tips dark brown to reddish brown, 8–14 × 3–6 mm.; viscid, aromatic, resinous, appressed.

ROCHESTER POPLAR

P. Maximowiczii (♀) × *P. nigra plantierensis* (♂)

SUMMER CHARACTERS. *Stems*. Round, sparsely puberulose, bright glossy green toward tip (growing tip tinged with red), duller slightly brownish green toward base; lenticels white and linear toward tip, greyish white and oval toward base, 0.5–3.5 mm. long. Secondary shoots more brownish green. *Leaves*. Broadly ovate to oval, apex very short acute, base cordate; firm, rather leathery, dull dark green above, glaucous below; margin medium finely crenate, usually 4–6 crenations per cm., glandular, undulate; midrib and large veins green above and below, somewhat sparsely puberulose above, more sparsely puberulose below. Petioles round, green (on young leaves very slightly tinged with red above), lenticellate, puberulose above, sparsely puberulose below, 25–38 mm. long. Stipules subulate, green fugaceous. Leaves on secondary shoots broadly elliptical, apex very short acute, base obtuse to slightly cordate; margin more finely crenate, usually 5–9 crenations per cm. *Buds*. Broadly lanceolate, sharp pointed, 8–12 mm. long, glossy, green to reddish brown, viscid, aromatic. Closely appressed except at tip of occasional bud.

WINTER CHARACTERS. *Stems*. Round, brown toward tip, olive brown and reticulate toward base. Lenticels pinkish, linear toward tip, elliptical or narrow oval toward base. Pith five-sided, brownish, homogeneous. Leaf-scars broadly triangular, decurrent, with three prominent bundle-scars. Stipule-scars linear to narrow V-shaped. *Buds*. Axillary buds broadly lanceolate with acute tips, brown, 12–16 × 3–7 mm.; viscid, resinous aromatic, closely appressed except at tip.

The four hybrids described above have *P. Maximowiczii* as a female parent and they strongly resemble this parent as do all the various hybrids which were obtained with this species. The GENEVA POPLAR and the OXFORD POPLAR have the same pollen parent, *P. berolinensis*, and they are very similar in appearance. They differ from the ANDROSCOGGIN POPLAR and the ROCHESTER POPLAR in the following characters: the leaves are somewhat more coarsely crenate; the midrib and veins are glabrous below; and the summer buds are usually slightly shorter. In the winter condition, the stems are slightly ridged toward the tip; the leaf-scars are winged and keeled by prominent, short, rounded ridges; and the stipule-scars are rather inconspicuous.

The ANDROSCOGGIN POPLAR is characterized by its more elliptical leaves; by the corky ridges toward the tip of the winter stems; and by the leaf-scars, which are winged and keeled by sharp, narrow, corky ridges extending to the next lower bud, except at the base of the stem.

The ROCHESTER POPLAR is the most easily distinguished of the four hybrids described above. The more noticeable differences are as follows. In the summer condition, the stems are bright glossy green and puberulose toward the tip, and slightly brownish green toward the base; the midrib and veins of the leaves are green above; and buds are quite green and the tips are occasionally slightly out-curved. In the winter condition the stems are round throughout with practically glabrous tips; the tips of the buds are more noticeably out-curved; and the leaf-scars are not prominently winged and keeled as are those of the other three hybrids.

The ANDROSCOGGIN POPLAR has been very slightly infected by *Melampsora* leaf rust, but there have been no injurious effects for the infection has taken place very late in the season, just prior to the leaf fall. The three other clones have been practically immune to this disease up to the present time.

STRATHGLASS POPLAR

P. nigra (♀) × *P. laurifolia* (♂)

SUMMER CHARACTERS. *Stems.* Round, very slightly ridged toward tip, sparsely puberulose except at base, reddish brown mottled with green above, olive or greyish green somewhat reticulate below; lenticels white and linear toward tip, oval and light brown to grey toward base, 0.5–5.0 mm. long. Secondary shoots round throughout, less red. *Leaves.* Ovate, apex acute or occasionally short acuminate, base obtuse or occasionally very slightly cordate at petiole, firm, medium green above, light green below; margin somewhat coarsely crenate, usually 2–4 crenations per cm., glandular, undulate; veins green above and below, basal portion of midrib

very sparsely puberulose above, glabrous below; petioles slightly flattened, bright red and puberulose above, less red to green and very sparsely puberulose below, lenticellate, 30–45 mm. long. Stipules triangular, sharp pointed, green, tardily fugaceous. Leaves of secondary shoots narrowly ovate to elliptical, apex acute to short acuminate, base obtuse to broadly cuneate; margin somewhat more finely crenate, usually 1–5 crenations per cm. Petioles often less red. *Buds*. Lanceolate, sharp pointed, 6–11 mm. long, glossy, dark brown, very slightly viscid, aromatic; rather loosely appressed.

WINTER CHARACTERS. *Stems*. Round, slightly ridged and greenish brown toward tip, greenish grey toward base; lenticels brownish grey, linear toward tip, oval to circular toward base; pith five-sided, light brown, homogeneous. Leaf-scars triangular to almost cordate, not very strongly decurrent, winged and keeled by rather narrow ridges, on upper portion of stem these ridges run to next lower bud; three prominent bundle-scars. Stipule-scars quite conspicuous, irregularly narrow V-shaped. *Buds*. Terminal buds broadly ovate. Axillary buds lanceolate with acute tips, 6–11×3–4 mm., reddish brown, not resinous or viscid, appressed.

FRYE POPLAR

P. nigra (♀) × *P. laurifolia* (♂)

SUMMER CHARACTERS. *Stems*. Round, with low, well defined ridges toward tip, sparsely puberulose except at base, reddish brown mottled with green above, olive or greyish green somewhat reticulate below. Lenticels linear and white above, oval and light brown or grey below, 0.5–4.0 mm. long. Secondary shoots round, usually not at all ridged, less red. *Leaves*. Ovate, apex acute, base slightly cordate; firm, medium green above, lighter green below; margin somewhat coarsely crenate, usually 3–4 crenations per cm., glandular, undulate; veins green above and below, midrib and basal portions of large veins puberulose or sparsely puberulose above, glabrous below. Petioles slightly flattened, dull red and puberulose above, green and glabrous below, 30–45 mm. long. Stipules triangular, sharp pointed, green, tardily fugaceous. Leaves of secondary shoots narrowly elliptical, apex acute, base obtuse to broadly cuneate; margin somewhat more finely crenate, usually 4–7 crenations per cm. *Buds*. Lanceolate, sharp pointed, 6–10 mm. long, dark brown, very slightly viscid, aromatic, appressed.

WINTER CHARACTERS. *Stems*. Round, slightly ridged on upper portion, reddish brown toward tip, brownish green toward base; lenticels brownish grey, linear toward tip, oval to circular toward base; pith five-sided, light

brown, homogeneous. Leaf-scars triangular to almost cordate, not very strongly decurrent, winged and keeled by rather narrow ridges, on upper portion of stem these ridges run to next lower bud; three prominent bundle-scars. Stipule-scars somewhat conspicuous, narrow half-crescent shaped. *Buds*. Terminal buds broadly ovate. Axillary buds lanceolate with acute tips, $6-11 \times 3-4$ mm., reddish brown, appressed, not resinous or viscid.

RUMFORD POPLAR

P. nigra (♀) \times *P. laurifolia* (♂)

SUMMER CHARACTERS. *Stems*. Upper portion ridged, round toward base, very sparsely puberulose (almost glabrous) above, glabrous at base, growing tips covered with a slightly viscid aromatic secretion; brownish green toward tip, greyish green somewhat reticulate toward base; lenticels linear and white or brownish white toward tip, oval to circular and greyish brown toward base, 0.5-4.0 mm. long. Secondary shoots much less ridged, almost round, almost glabrous. *Leaves*. Broadly ovate, apex acute, base slightly cordate; firm, dark glossy green above, lighter green below; margin rather coarsely crenate, usually 2-2.5 crenations per cm., glandular; veins green above and below, midrib very sparsely puberulose on upper side toward base, glabrous below. Petiole flattened, oval, tinged with red and very sparsely puberulose above, green and practically glabrous below, lenticellate, 50-65 mm. long. Stipules broadly subulate, apex narrow acuminate, tardily fugaceous. Leaves of secondary shoots narrow ovate, apex acute, base rounded to acute; margin somewhat more finely crenate, usually 3-4 crenations per cm. *Buds*. Lanceolate, 6-10 mm. long, somewhat glossy, dark brown, slightly viscid, aromatic, appressed.

WINTER CHARACTERS. *Stems*. Rather strongly ridged toward tip, round toward base; greenish brown toward tip, olive green and coarsely reticulate toward base; lenticels brownish to pinkish white, linear toward tip, elliptical to circular toward base; pith five-sided, brown, homogeneous. Leaf-scars triangular with apex sufficiently long to appear more or less equilateral, decurrent, winged and keeled by narrow ridges which extend to the next lower bud except near base of stem, three prominent bundle-scars. Stipule-scars prominent, curved-linear to narrow half-crescent shaped, usually blackened. *Buds*. Terminal buds broadly ovate. Axillary buds lanceolate with narrow acute tips, $9-12 \times 3-5$ mm., dark brown, basal scale somewhat lighter brown; very slightly resinous, not viscid, somewhat aromatic, appressed.

The STRATHGLASS POPLAR, the FRYE POPLAR, and the RUMFORD POPLAR are sister seedlings of *P. nigra* \times *P. laurifolia*. A total of 377 seed-

lings of this cross were grown and 10 were included in the first selections of which these were later considered to be somewhat superior. All these seedlings were very uniform in general characters and appearance, with resinous buds and leaves somewhat glaucous beneath. They resemble the balsam male parent more than the female parent *P. nigra*. The three seedlings here described are to be identified and distinguished by several characters. In comparison with the STRATHGLASS POPLAR, the stems of the FRYE POPLAR are somewhat more strongly ridged toward the tip; the leaves are a trifle more cordate; the petioles are a duller red above; the upper side of the mid-rib and veins are slightly more puberulose; and the winter stems are generally a trifle more reddish or brownish colored. The STRATHGLASS POPLAR also develops fewer lateral branches than the FRYE POPLAR in nursery stock. The STRATHGLASS POPLAR is distinct from the RUMFORD POPLAR in its more persistent and less strongly keeled stipules, in the less appressed but more acuminate buds, and in its somewhat smaller leaves.

The RUMFORD POPLAR differs from its named sister hybrids in its more sparsely puberulose, almost glabrous, stems and in its darker green, broadly ovate leaves with acute apex and slightly cordate base. The petioles of the leaves are also longer and the margin is hardly undulate. The leaf-scars on winter stems are somewhat more decurrent and have a more strongly elongated apex.

ROXBURY POPLAR

P. nigra (♀) × *P. trichocarpa* (♂)

SUMMER CHARACTERS. *Stems.* Round, with low narrow ridges on upper portion, on occasional stems, ridges persisting toward base; sparsely puberulose, end of growing tip somewhat viscid and aromatic, reddish brown toward tip, greyish brown reticulate toward base; lenticels white and linear above, oval and light brown below, 0.5-4.5 mm. long. Secondary shoots round throughout. *Leaves.* Broadly ovate to oval, apex acute to short acuminate, base obtuse; firm somewhat glossy medium green above, light green below; margin quite coarsely crenate, usually 2-3 crenations per cm., glandular, somewhat undulate; veins green above and below, mid-rib very sparsely puberulose (almost glabrous) toward base, glabrous below. Petioles flattened, bright red and puberulose above (older leaves less bright red), green and practically glabrous below, 30-50 mm. long. Stipules subulate, sharp pointed, green, youngest with brownish tips, tardily fugaceous. Leaves of secondary shoots ovate to elliptical, apex acute to short acuminate, base obtuse to broadly cuncate, margin somewhat more finely crenate, usually 3-5 crenations per cm. *Buds.* Lanceolate, 6-10 mm. long, glossy, dark brown, viscid, aromatic, appressed.

WINTER CHARACTERS. *Stems.* Rather strongly ridged toward tip, round toward base, brown toward tip, grey toward base; lenticels greyish white, somewhat broadly linear toward tip, elliptical to circular toward base; pith five-sided, brown, homogeneous. Leaf-scars triangular, with strongly elongated apex, not strongly decurrent, winged and keeled by rather sharp ridges which extend to the next lower bud, three prominent bundle-scars. Stipule-scars prominent, narrow V-shaped usually slightly curved and blackened. *Buds.* Terminal buds broadly ovate. Axillary buds broadly lanceolate with short acuminate tips, 9-14×4-6 mm., rather dark brown, basal scale light brown. Somewhat resinous, viscid, aromatic, appressed.

The ROXBURY POPLAR can be distinguished from the three hybrids, the STRATHGLASS, FRYE, and RUMFORD POPLARS, which are of *P. nigra* × *P. laurifolia*, by its more brown-colored stems, broadly ovate to oval leaves, with acute to acuminate apex, cordate base and slightly undulate margin, and its more broadly lanceolate winter buds.

The ROXBURY POPLAR can be distinguished from the STRATHGLASS POPLAR and the FRYE POPLAR by its somewhat more flattened petioles; somewhat longer and darker brown winter buds; and leaf-scars with more elongated apex. The ROXBURY POPLAR differs from the RUMFORD POPLAR in having a more undulate leaf margin; less sparsely puberulose and brighter red petioles; and winter stems less strongly ridged at the tips. Further, the base of the stem of the RUMFORD POPLAR is coarsely reticulate.

ANDOVER POPLAR

P. nigra betulaefolia (♀) × *P. trichocarpa* (♂)

The female parent, *P. nigra betulaefolia*, is a variety of the European black poplar. This hybrid has been slightly susceptible to *Melampsora* rust but up to the present time it has not been injured by the disease.

SUMMER CHARACTERS. *Stems.* Round, with low, narrow ridges toward tip, very sparsely puberulose, growing tip viscid, aromatic; reddish brown toward tip, light olive green somewhat reticulate toward base; lenticels white and linear toward tip, light brown and oval toward base, 0.5-4.0 mm. long. Secondary shoots round, more densely puberulose. *Leaves.* Broadly ovate, apex acute, base cordate; firm, somewhat glossy medium green above, lighter green below; margin rather coarsely crenate, usually 2-3 crenations per cm., glandular, somewhat undulate; midrib and large veins green and sparsely puberulose above, green and glabrous below. Petioles flattened, in young leaves bright red with narrow strip of green on under side, in old leaves green with slight red on upper side, sparsely pu-

berulose, 30–50 mm. long. Stipules subulate, rather long pointed, green, fugaceous. Leaves of secondary shoots ovate, apex acute to acute-acuminate, base obtuse; margin somewhat more finely crenate, usually 4–5 crenations per cm. *Buds*. Narrow lanceolate, sharp pointed, 6–10 mm. long, glossy dark brown, somewhat viscid, aromatic, appressed.

WINTER CHARACTERS. *Stems*. Somewhat ridged and greyish brown toward tip, round and brownish grey toward base; lenticels greyish white, rather broadly linear toward tip, elliptical to circular toward base; pith five-sided toward tip, round toward base, light brown, homogeneous. Leaf-scars broadly triangular, decurrent, winged and keeled by rather sharp low ridges, which are rounded at the leaf-scar and extend to the next lower bud except at base of stem; three prominent bundle-scars. Stipule-scars prominent, linear, usually curved and blackened. *Buds*. Terminal buds broadly ovate. Axillary buds somewhat broadly lanceolate with narrow acute tips, 9–12×4–5 mm., light chestnut brown, slightly resinous, not viscid, somewhat aromatic, appressed.

MAINE POPLAR

P. tacamahacca candicans clone BALM OF GILEAD (♀) × *P. berolinensis* (♂)

Both parents of this hybrid are members of the balsam poplar group. The BALM OF GILEAD is a variant of the northern balsam poplar, *P. tacamahacca*, which has been cultivated as a clone.

SUMMER CHARACTERS. *Stems*. Round, most vigorous shoots very slightly ridged toward tip, puberulose practically to base, greenish brown toward tip, greyish green somewhat reticulate toward base; lenticels white and linear toward tip, oval and brownish (or pinkish) white toward base, 0.5–5.0 mm. long. Secondary shoots round, not at all ridged, less sparsely puberulose, more red. *Leaves*. Ovate, apex acute, occasionally almost acuminate, base obtuse to slightly cordate, firm, medium green above, light green below; margin rather coarsely crenate, usually 2–3 crenations per cm., glandular, undulate; veins green above and below, midrib and lower parts of large veins sparsely puberulose above, extremely sparse below. Petiole slightly flattened, rather bright red and puberulose above, green and very sparsely puberulose below, lenticellate, 32–44 mm. long. Stipules subulate, green, sharp pointed, fugaceous. Leaves of secondary shoots ovate to narrow ovate, apex acute, base obtuse to cuncate; margin a trifle more finely crenate, usually 3–5 crenations per cm.; veins usually more sparsely puberulose. *Buds*. Lanceolate, sharp pointed, 6–10 mm. long, glossy, dark brown, somewhat viscid, aromatic, appressed.

WINTER CHARACTERS. *Stems*. Round, slightly ridged and brownish grey

toward tip, grey toward base; lenticels greyish white, rather broadly linear toward tip, elliptical to circular toward base; pith five-sided, brown, homogeneous. Leaf-scars very broadly triangular, somewhat decurrent, winged and keeled by low ridges, these ridges are rather sharp except at the leaf-scar where they are rounded, and on upper portion of stem they extend to the next lower bud. Three prominent bundle-scars. Stipule-scars prominent, narrow half-crescent shaped, usually more or less blackened toward base of stem. *Buds*. Terminal buds broadly ovate. Axillary buds, broadly lanceolate with slightly acuminate tips, 9-14×4-6 mm., medium brown, appressed, not resinous or viscid.

SUMMARY OF THE SELECTIONS

The parentage of the ten hybrids here described, the number of sister seedlings grown, and the number which were propagated as first selections are summarized in the following tabulation. The parents which are in the black poplar group are indicated by *italics* and those of the balsam group by **bold face** type.

SELECTION HYBRIDS	PARENTAGE		TOTAL NO. SEEDLINGS	NUMBER OF 1ST SELECTIONS
FRYE	<i>P. nigra</i>	× <i>P. laurifolia</i>	377	10
RUMFORD	"	× "	"	"
STRATHGLASS	"	× "	"	"
ROXBURY	"	× <i>P. trichocarpa</i>	200	3
ANDOVER	<i>P. nigra betulifolia</i>	× "	209	1
GENEVA	<i>P. Maximowiczii</i>	× <i>P. berolinensis</i>	112	8
OXFORD	"	× "	"	"
ROCHESTER	"	× <i>P. nigra plantierensis</i>	145	1
ANDROSCOGGIN	"	× <i>P. trichocarpa</i>	5	3
MAINE	<i>P. Balm of Gilead</i>	× <i>P. berolinensis</i>	82	2

It will be noted that the FRYE, RUMFORD, STRATHGLASS, ROXBURY, ANDOVER and ROCHESTER POPLARS are hybrids between *P. nigra* or its varieties *betulifolia* and *plantierensis* and some member of the balsam group. The other four have balsam poplars for both parents. Nine different plants are involved as parents: three are black poplars; six are balsam poplars.

The ten hybrids which are now named represent but a small part of the hybridizations which were made and of which seedlings were grown. Thirty-four different poplars were employed as parents, including 3 white poplars, 5 aspens, 17 black poplars, and 9 balsam poplars. About 13,000 seedlings were grown. Of the various groups of hybrids, 27 were most

promising; for these the total number of seedlings grown was 4,309; the number in a group of sister seedlings ranged from 5 to 705, and there were 14 groups of 200 or more each. From each of these 27 groups, "first selections" were made of from one to ten plants (a total of 69 seedlings) for propagation and special study.

Further selection among these seedlings was based on performance in Maine, chiefly while under nursery propagation. Final judgement of individual merit must be based on behavior in forest plantations. These ten hybrids may not be the best of the seedlings for use in warmer climates where the growing season is longer than in Maine. A test planting of the 69 first selections is now being grown at Strathglass Farm near New York City where the season of growth is several weeks longer than in Maine and where there was slight rust during 1932 and 1933. Here several others of the first selections have surpassed the named hybrids in vigor of growth. It may be mentioned that certain of the hybrids which were most vigorous during the early years of this study later suffered severe injury from the *Melampsora* rust. This was especially true of hybrids having *P. balsamifera virginiana* and *P. trichocarpa* as the parents. Various hybrids of the white poplar group which show vigorous growth may be of value for the production of match wood, which is a feature in reforestation efforts in various sections in Europe. Certain hybrids with columnar habits of growth and others of dwarf size may have value as ornamentals.

Since cuttings of these ten hybrid clones are being distributed rather widely for trial culture, it may be well to state that the above descriptions are the first that have been published for these individual clones and also that herbarium specimens and photographs of them have been deposited in the herbarium of The New York Botanical Garden.

INDEX TO AMERICAN BOTANICAL LITERATURE 1931-1934

The aim of this Index is to include all current botanical literature written by Americans, published in America, or based upon American material; the word America being used in the broadest sense.

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Biological specialization in *Darluca filum*

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(WITH PLATE 25)

The genus *Darluca* comprises a comparatively small group of parasitic fungi of the Family Sphaeropsidaceae of the Order Sphaeropsidales of the Class Fungi Imperfecti. It was named in honor of Michel Darluc, a French doctor of medicine. The members of the genus are well known as parasitizing the rust fungi, occurring on a variety of rusts scattered throughout the entire world. The present paper deals with the results of greenhouse cultures of *Darluca filum* (Biv.) Cast. on various rusts. The writer (Keener, 1933) has already reported the results of laboratory studies with pure cultures of this species of *Darluca*.

HISTORICAL

The most common and widely distributed species of the genus is *Darluca filum* which was first described as *Sphaeria filum* by Bivona-Bernardi in the year 1813. In 1823 Fries described the same fungus as *Phoma filum*. The generic name *Darluca* was established by Castagne in 1851, who cited *Sphaeria filum* as the type species. Previous to this time the same fungus had already been sent out from the Castagne herbarium to several European botanists as *Darluca vagans*, so that in reality the generic name was in limited use sometime before the actual founding of the genus. Since the name *Darluca vagans* as used by Castagne previous to 1851, was not published, it cannot be recognized under any system of nomenclature as valid. On the other hand, *Phoma filum* of Fries, in view of its proper publication, does become a synonym of *Darluca filum* (Biv.) Cast.

Darluca filum has been reported on both macro-cyclic and micro-cyclic rusts, and as occurring on the aecial, uredinal, and telial sori, but more commonly on the uredinia. Among the rusts on which this species has been reported, are representatives of such genera as *Aecidium*, *Cerotelium*, *Chrysomyxa*, *Coleosporium*, *Frommea*, *Kuehneola*, *Melampsora*, *Peridermium*, *Phragmidium*, *Pileolaria*, *Puccinia*, *Ravenelia*, *Tranzschelia*, *Uromyces*, *Uromycladium*, and *Uredo*. In addition the author has observed *D. filum* on the rust genera *Gymnoconia* and *Phakopsora*. The examples

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below will give some idea as to the variety of rusts on which this species of *Darluca* has been found. In 1851 Castagne reported *D. filum* (as *D. vagans*) on *Pileolaria Terebinthi*. In 1897 Cobb reported *D. filum* on *Tranzschelia punctata*. In 1906 McAlpine reported *D. filum* on several Australian rusts among which were *Uromycladium simplex*, two species of *Aecidium*, and *Phragmidium Potentillae*. In 1913 it was listed by Lind on *Chrysomyxa Abietis*. In 1920 Adams reported *D. filum* on *Peridermium Peckii* (*Pucciniastrum Myrtilli*) on *Tsuga canadensis* and on *Coleosporium delicatulum* on *Euthamia tenuifolia*. So far as is known, this was the first report of its occurrence on a Gymnosperm rust. In 1929 Moss found *D. filum* on *Melampsora confluens* on *Salix lutea*. In 1930 Sydow (p. 186) reported it on *Ravenelia spinulosa* on *Cassia biflora* in South America. During the same year, Stevens (1930a) reported *D. filum* as occurring on *Uredo Dioscoreae* on *Dioscorea* sp. in Panama. In 1931 Petrak and Ciferri reported the parasite on *Ravenelia Ingae* on *Inga vera*. It has been said by some authors that *D. filum* attacks at least twenty-four percent of the species of the rust genus *Puccinia* and almost as high a percentage of the species of the genus *Uromyces*.

Darluca filum has also been reported in association with fungi other than the Rusts. In 1930 (b) Stevens listed *D. filum* in association with the fungus *Diplodia uredinicola*. Previous to this report, Castagne (1851) had listed *D. filum* (as *D. vagans*) on *Diplodia perpusilla*.

Since the founding of the genus there have been described in the literature approximately twenty-five other species of *Darluca*. Various reports have listed these primarily as rust parasites, although a few species have been listed as occurring directly on phanerogamic hosts, or in association with fungi other than the rusts. For those species recorded as occurring directly on phanerogamic hosts, unassociated with rusts, Clements and Shear (1931) have proposed the creation of the new genus *Darlucis*. Accordingly, the genus *Darluca* would thus be split up into two genera, retaining the name *Darluca* for those species parasitic on rusts.

A perfect stage in the *Darluca* life history has been established under the generic name *Eudarluca* by Spegazzini in 1908. This genus is classed in the Family Sphaeriaceae of the Order Sphaeriales of the Class Ascomycetes. When Spegazzini set up the genus he described *Eudarluca australis* as the type species. In 1910 the same author described *Darluca australis* which apparently he thought was the imperfect stage of *Eudarluca australis* Speg. although an actual connection between the two stages has undoubtedly never been established by cultural methods. So far as can be determined from the literature, there has been only one other species of *Eudarluca* described, namely, *Eudarluca venezuelana*, reported

by Sydow (1930, p. 71) from Venezuela, South America. In the same report, Sydow (1930, p. 186) described an imperfect form, *Darluca venezuelana*, and here again it is apparent that there is merely an assumed connection between the imperfect or *Darluca* stage and the perfect or *Eudarluca* stage. The writer has observed an ascomycete on *Puccinia obscura* on *Juncoides campestre* and on *Puccinia Peckii* on *Carex normalis*, in Pennsylvania, which he believes may be a perfect stage of *Darluca filum*. Inoculations with this fungus as well as with the imperfect forms are herein reported.²

Probably the first attempt at culture work with any *Darluca* species was made by Sappin-Trouffy in the year 1896. This worker germinated *Darluca* conidia in contact with rust spores on the surface of water cultures in the laboratory. He observed that upon germination, the conidia sent hyphal strands into the urediniospores and teliospores of the rusts concerned. In an article concerning *Darluca* on *Uromyces caryophyllinus* and on *Puccinia Asparagi*, Blodgett (1907-1908) suggested that the fungus might be cultured by spraying conidial suspensions on rusted carnation and asparagus plants in the greenhouse.

MORPHOLOGY OF THE FUNGUS

During its development in the field, *D. filum* produces small, globose or elongated, black, shiny, fruiting bodies known as pycnidia. These structures vary considerably in size, are slightly beaked, and are sometimes fused at their bases or along the pycnidial wall (Pl. 25, figs. 4, 6, 7). They possess distinct ostiolar openings, through which the spores are exuded in thin, white or grayish, mucilaginous, threads. The ostiolar papillae become somewhat depressed as the pycnidia mature.

The pycnidia occur either singly or in groups, within or upon the rust sorus (Pl. 25, figs. 1-8), and rarely directly on the host plant epidermis. In the latter case, the fungus is probably parasitic on the rust hyphae concealed within the tissues of the host plant.

The spores are produced in great numbers within the pycnidial cavity (Pl. 25, fig. 5). Adams (1920, fig. 4) although not discussing such a process, shows that the conidia are produced singly and acrogenously, at

² Isolation No. 11-1 was made from conidia and isolations No. 11-2 and 12-2 from the ascospores of this fungus. Inoculation results with isolations No. 11-1 and 12-2 are herein recorded. The fungus shows all the characteristics of *Darluca filum*, but until further studies have been completed, the author has reserved his opinion as to the exact status of this form. For the present it will suffice to say that isolations No. 11-1 and 12-2 may be looked upon as constituting strains of the common species *D. filum*.

the tips of short pedicel-like hyphal processes sent out from the cells of the pycnidial wall; a single wall cell producing but one hyphal process with a spore at its tip (Pl. 25, figs. 5, 6). Such a method of spore production is not uncommon in certain members of the Order Sphaeropsidales to which *Darluca* belongs. The conidia are 2-celled, sometimes 3-4-celled, hyaline, oblong to fusoid, $10-21 \times 3-7\mu$, slightly constricted at the septa, and when young possess two apiculate spine-like projections at each end of the spore. These spines tend to disappear as the spores become mature. As a result of this tendency for the older spores to lose their spines, Adams (1920) was led to conclude that there might possibly exist two distinct strains of the parasite; one with spines on the spores and the other lacking such spore structures.

In the generic description published in 1851, Castagne made no mention of spines on the spores of his specimen. So far as can be determined from the literature, the first mention of these structures was by Saccardo in 1884.

OBJECT

The object of this work was to determine whether or not biological specialization exists within the species *Darluca filum*, and if so, the extent to which it occurs. An indication of specialization was secured from the author's previous study of several isolations and their behavior on various laboratory culture media (Keener, 1933)

MATERIALS AND METHODS

Pure cultures of isolations from conidia of *D. filum* as well as from ascospores of the fungus found on *Puccinia obscura* and on *Puccinia Peckii*, were used as a source of spore inoculum with which to conduct the greenhouse cultures. Each isolation was considered a strain and the two terms are hereafter used interchangeably.

The author (Keener, 1933) has already described the methods used in isolating from conidia and ascospores. By the use of these methods, thirteen isolations (11 from conidia and 2 from ascospores), from 11 different rusts (7 of the rust genus *Puccinia* and 4 of the genus *Uromyces*), on 10 different phanerogamic hosts, collected in 3 states, were made. The isolations with their arbitrary pure culture numbers and their sources, are given in the table on the next page.

The culture work conducted in the greenhouse can be conveniently divided into two phases, (1) the culturing of the various rusts on their phanerogamic hosts, and (2) the culturing of the various *Darluca* isolations on these rusts.

Rust cultures. In order to obtain suitable material to be inoculated with *D. filum*, healthy plants had to first be infected with various rusts. To secure rust infections the atomizer, hypodermic syringe, and scalpel methods were employed. The first method proved to be the most satisfactory for the majority of rusts used. For each inoculation, a suspension of

PURE CULTURE NUMBER	SOURCE		
	NAME OF RUST HOST	PHANEROGAMIC HOST	STATE
1	<i>Puccinia Sorghi</i>	<i>Zea Mays</i>	Pennsylvania
2	<i>Puccinia poeciliformis</i>	<i>Phleum pratense</i>	Pennsylvania
3	<i>Uromyces Silphii</i>	<i>Juncus tenuis</i>	Pennsylvania
3-B	<i>Uromyces Silphii</i>	<i>Juncus tenuis</i>	West Virginia
4	<i>Uromyces fallens</i>	<i>Trifolium pratense</i>	New Jersey
5	<i>Puccinia hibisciata</i>	<i>Muhlenbergia Schreberi</i>	New Jersey
6	<i>Puccinia Violae</i>	<i>Viola</i> sp.	New Jersey
7	<i>Uromyces Polygoni</i>	<i>Polygonum aviculare</i>	Pennsylvania
8	<i>Uromyces Junci-effusi</i>	<i>Juncus effusus</i>	Pennsylvania
9	<i>Puccinia Hieracii</i>	<i>Leontodon Taraxacum</i>	Pennsylvania
*11-1	<i>Puccinia obscura</i>	<i>Juncoides campestre</i>	Pennsylvania
**11-2	<i>Puccinia obscura</i>	<i>Juncoides campestre</i>	Pennsylvania
**12-2	<i>Puccinia Peckii</i>	<i>Carex normalis</i>	Pennsylvania

* Isolated from conidia.

** Isolated from ascospores.

urediniospores in distilled water was prepared in the bowl of an atomizer, and was then sprayed onto healthy, potted plants in the greenhouse. Plants so treated were then placed in sand pits and covered with bell jars. The bell jars were removed in from 24 to 48 hours.

In their studies on smut resistance in corn seedlings grown in the greenhouse, Tisdale and Johnston (1926) found that the use of a hypodermic syringe proved to be a highly satisfactory means of inoculation. Inoculations made in this manner were most satisfactory for securing a good distribution of rust sori on both corn and bean plants. In using this method, a suspension of urediniospores in distilled water was drawn up into the tube of a hypodermic syringe, and was then injected into healthy plants, at the ground level. When this method is used, bell jars can be dispensed with. The rust infections which resulted spread to even the uppermost leaves of the host plants.

In the majority of cases, urediniospores were the inoculum used. In the case of *Puccinia Malvacearum* the inoculum consisted necessarily of basidiospores. To secure infections with this rust, heavily rusted holly-hock leaves were brought in from the field and were supported on wire

gauzes over healthy, potted plants in the greenhouse. The inoculated plants were then covered with bell jars as before.

In a few instances, such as for *Kuehneola Uredinis* on *Rubus allegheniensis*, *Puccinia Anemones-virginianae* on *Anemone virginiana*, *Puccinia Circaeae* on *Circaea alpina*, and *Uromyces verruculosus* on *Lychnis alba*, it was necessary to depend on plants that had become naturally rusted under field conditions. Such plants were dug up, were transplanted in separate pots in the greenhouse and allowed to stand for two weeks, in order to be certain that no stray infections of *Darluca* from the field were present. A final examination was given these plants before inoculating them with the various *Darluca* isolations.

Darluca cultures. In making inoculations onto rusts with the various isolations of *D. filum*, a suspension of conidia from pure cultures was prepared, and an atomizer was then employed to spray the suspensions on rusted plants in the greenhouse. Each time an inoculation was made, a bell jar was placed over the inoculated plant for a period of from 24 to 48 hours. Results of inoculations were observed in from 6 to 9 days after the initial set-up.

After each series of inoculations, bell jars were sterilized by spraying them with a 20% formaldehyde solution. The sand used in the pits was discarded, and the pits themselves sterilized by spraying them with the same percent formaldehyde. Atomizers were sterilized after each inoculation, by submerging them in 70% alcohol for from 12 to 24 hours.

Altogether nineteen rust species representative of five genera were employed as hosts for the isolations of *Darluca*. Among these were two species of the genus *Coleosporium*, one of *Frommea*, one of *Kuehneola*, eleven of *Puccinia*, and four of *Uromyces*. Of these, three were micro-cyclic, while sixteen were macro-cyclic species. In some instances it was impossible to inoculate all nineteen rusts with a given isolation of the parasite. This was due either to insufficient rust material or to the dying off of certain phanerogamic hosts in the greenhouse.

In view of the fact that particular rust species used in this work occur naturally on more than one phanerogamic host, a more complete citation of hosts for such rusts precedes Table 1.

RESULTS

Isolations No. 9 and 11-2 failed to produce sufficient spores with which to conduct greenhouse inoculations.

Table 1 shows the varied reactions of eleven of the thirteen isolations of *D. filum* on the nineteen rust species. Wherever blanks occur, they indicate that no inoculation of the particular strain on the rust species

concerned, was made. In Table 1 the fractions were determined as $\text{Ratio} = \frac{\text{Number of rusted leaves inoculated}}{\text{Number of rusted leaves infected}}$. Zeros in the denominators therefore indicate negative inoculation results. The average percentage of infection figures in the last column of the table, show the relative susceptibility of any one of the nineteen rusts to the eleven isolations of the parasite. The lowermost row of average percentage of infection figures, show the relative virulence of any particular isolation on the nineteen rust species. Average percentage of infection was determined as, $\text{Average percentage of infection} = \frac{\text{Number of leaves infected}}{\text{Number of leaves inoculated}} \times 100$. The table, therefore, shows the inoculation results not only from a simple positive and negative standpoint, as determined from the ratios, but also from the average percentage of infection standpoint, as determined, from the figures in the last column and the lowermost row.

TABLE 1
*Relative susceptibility of nineteen rusts and relative virulence of eleven isolations of Darluca filum.**

NAME OF RUST HOST	ISOLATION NUMBER											AV. % INF.
	1	2	3	3 B	4	5	6	7	8	11 1	12 2	
Coleosporium Campanulae	3/0		3/0		7/0							0
Coleosporium Solidaginis	5/0	5/0	6/3	9/0	7/0	8/0	4/1	8/0	9/0		4/0	6
Frommea obtusa	6/0	4/3	4/0	2/2	1/1	1/1	2/2	2/0	6/2	2/1	3/2	42
Kuehneola Uredinis	21/10	3/0	5/3	8/0	8/0	18/9	12/0	4/0	10/0	10/0	6/0	21
Puccinia Acetosae	6/4	28/8	9/0	10/0	10/0	10/5	8/3	6/4	4/2			42
Puccinia Anemones- virginianae	3/3	4/4	4/3	4/0	4/4	2/1	5/0	5/0	6/2	3/3	2/0	79
Puccinia Antirrhini	30/6	38/11	12/0	46/11	20/0	21/12	34/13	6/6	19/9	12/10	14/8	34
Puccinia Circaeae	5/5	6/0	3/3	6/3	10/6	7/5	6/0	7/0	6/3	10/5	4/3	47
Puccinia Clematidis	5/4	3/3	4/0	4/0	5/0	5/0	4/0	4/3	12/0	5/1	3/0	20
Puccinia Hieracii	30/10	4/1	10/6	34/0	6/3	14/8	12/0		17/11			31
Puccinia Malvacearum	4/0				7/0			4/0	3/2			11
Puccinia Menthae	21/13	12/0	8/0	18/0	22/0	30/21	15/10	10/0	59/40	44/26	19/14	50
Puccinia poculiformis (1)	10/0	35/23	25/0	27/0	29/11	33/30	28/21	25/0				40
Puccinia poculiformis (2)	3/0	8/0	3/0	8/0	7/0	4/0	3/0	10/0	3/0			0
Puccinia Sorghi	20/12	30/15	25/20	25/5	7/4	23/19	17/11	10/9	21/12	10/6	12/6	60
Uromyces appendiculatus	14/10	10/5	7/0	14/0	7/4	17/5	12/4	10/8	7/4	14/0	11/5	37
Uromyces caryophyllinus	5/0			17/5		9/9			15/7	8/3		44
Uromyces Polygoni	14/11	30/29	10/6	14/8	24/15	12/0	17/13	21/14	42/21	13/0	13/10	60
Uromyces verruculosus	5/0	14/11	34/15	6/0	21/5	11/7	36/0		9/0		20/10	31
Av. Percentage of Infection	42	48	34	13	26	59	36	33	46	42	52	×

* Ratio = $\frac{\text{No. of leaves inoculated}}{\text{No. of leaves infected}}$

Average percentage of infection = $\frac{\text{No. rusted leaves infected}}{\text{No. rusted leaves inoculated}} \times 100$.

In Table 1, *Coleosporium Campanulae* represents the uredinial stage of that rust on *Campanula rapunculoides* and *Coleosporium Solidaginis* the uredinial stage on *Solidago* sp. The host for *Puccinia Clematidis* was *Triticum aestivum*, while *Puccinia poculiformis* (1) had *Phleum pratense* for its host, and *Puccinia poculiformis* (2) was on *Poa compressa*. *Puccinia Hieracii* was used on *Leontodon Taraxacum* and *Puccinia Malvacearum* on *Althaea rosea*. *Puccinia Menihae* was on *Mentha spicata* while *Uromyces Polygoni* was on *Polygonum aviculare*. Golden Bantam sweet corn was employed as the host for *Puccinia Sorghi*, and Early Dawn carnation was used as the host for *Uromyces caryophyllinus*. For *Uromyces appendiculatus* the host was *Phaseolus vulgaris* var. "Kentucky Wonder." *Frommea obtusa* was on *Potentilla canadensis* and *Puccinia Acetosae* was on *Rumex Acetosella*, and *Puccinia Antirrhini* was on *Antirrhinum majus*. Other hosts have been previously mentioned.

DISCUSSION AND CONCLUSIONS

The writer's previous studies (Keener, 1933) of ten isolations of *Darluca filum* on various laboratory media, indicated the presence of six distinct strains of the rust parasite. However, the results of greenhouse cultures with eleven of thirteen isolations herein discussed, show that it is better to consider each isolation a distinct strain of *D. filum*.

In the remarks to follow, several terms are used which it seems advisable to define:

(1) Susceptibility: that quality of a host allowing for a successful attack by the parasite. As herein employed it denotes that quality of the various rust hosts which permitted them to be attacked by the various isolations. The degree of susceptibility of one rust as compared with another under similar conditions may be regarded as relative susceptibility, and may be roughly determined by comparing the average percentage of infection figures in the last column of Table 1.

(2) Virulence: that quality of the parasite which enables it to attack a host successfully. As mentioned herein it is used to denote that quality of the various *Darluca* isolations which enabled them to attack the various rusts. The degree or intensity of virulence of one isolation as compared with another under similar conditions may be looked upon as relative virulence, and in this instance may be determined by comparing the average percentage of infection figures in the bottom row of Table 1.

By the use of previously described methods, 175 inoculations with eleven of the thirteen isolations of *D. filum*, were completed. Of this number 101 or 58% proved to be positive and 74 or 42% gave negative results. Of the 175 inoculations, 149 were made on the 16 macro-cyclic (long-

cycled), and 26 on the 3 micro-cyclic (short-cycled) rust species. Of the 149 inoculations onto the 16 macro-cyclic rusts, 85 or 57% gave positive results and 64 were negative. The 26 inoculations onto the 3 micro-cyclic rusts resulted in 16 or 62% positive and 10 negative trials.

Considering the results from the standpoint of the rusts and also from that of the isolations, it will be noted that there are two possible ways of interpreting the specialization that is shown:

(1) The nineteen rusts varied in their susceptibility to the different isolations. Some rusts were more susceptible than others and some were non-susceptible.

(2) The eleven isolations proved variable in their virulence on the nineteen rusts. Any single isolation was more virulent on some rusts than it was on others.

From the standpoint of susceptibility to all isolations tested, the ratios in Table 1 show that *Puccinia Sorghi* was the most susceptible rust since it was successfully attacked by all of the isolations. *Puccinia Antirrhini* and *Uromyces Polygoni* were somewhat less susceptible, being attacked by only nine of the eleven strains, while *Frommea obtusa* and *Uromyces appendiculatus* were attacked by eight of the eleven isolations. Table 1 also shows that *Coleosporium Solidaginis*, *Kuehneola Uredinis*, and *Puccinia Clematidis*, all proved relatively non-susceptible to attack, and *Puccinia poculiformis* (2) was non-susceptible to all of the isolations.

If the average percentage of infection is considered an index of rust susceptibility, by comparing the figures shown in the last column of Table 1, it will be seen that *Puccinia Anemones-virginianae*, *Uromyces Polygoni*, and *Puccinia Sorghi* were the most susceptible, *Coleosporium Solidaginis* relatively non-susceptible, and *Puccinia poculiformis* (2) was entirely non-susceptible to attack. The average percentage of infection figures probably present a truer picture of relative susceptibility, than do the ratios indicating merely positive and negative results, as the former take into account not only the number of trials but also the actual number of rusted leaves inoculated.

Table 1 shows that the nineteen rusts varied in their susceptibility to any single isolation. For example, it will be observed that *Puccinia Anemones-virginianae*, *Puccinia Clematidis*, and *Uromyces Polygoni* were relatively the most susceptible, *Frommea obtusa* and *Puccinia poculiformis* (2) were less susceptible, and that *Coleosporium Solidaginis*, *Kuehneola Uredinis*, and *Puccinia Circaeae* were non-susceptible to the attacks of isolation No. 2. In the case of isolation No. 8, by the same sort of comparison it will be noted that *Puccinia Menihae* and *Puccinia Malvacearum* were most susceptible to the attacks of this isolation, while *Coleosporium Solida-*

ginis, *Kuehneola Uredinis*, and *Puccinia Clematidis* were non-susceptible. Comparing these results it will be seen that the rust species which appear to be most susceptible to isolation No. 2, are not necessarily the same species that seem to be most susceptible to isolation No. 8. The same is true of other examples.

Interpreting the specialization that is shown from the standpoint of the virulence of the *Darluca* isolations, it will be noted that some isolations seemed to be more virulent than others on the nineteen rusts. As shown in Table 1, isolation No. 5 was the most virulent, attacking successfully thirteen of seventeen rusts inoculated. Isolation No. 8 was somewhat less virulent attacking but twelve of seventeen rusts inoculated. Isolation No. 7 attacked only six out of fifteen, and isolation No. 3-B attacked six out of seventeen rusts, the latter two strains appearing to be the least virulent of the eleven.

If the relative virulence of the different isolations are determined from the average percentage of infection figures in the last row of Table 1, it will be noted that isolation No. 5 can still be regarded as being the most virulent on the nineteen rusts. Isolations No. 12-2 and 2 were somewhat less virulent while isolations No. 3-B and 4 proved to be the least virulent of the eleven.

It has been already pointed out that *Puccinia Anemones-virginianae*, *Puccinia Clematidis*, and *Uromyces Polygoni* may be considered as being the most susceptible of the nineteen rusts to isolation No. 2. It is also possible to consider that isolation No. 2 was more virulent on these three rusts, less virulent on *Frommea obtusa* and *Puccinia poculiformis* (1) while it was not able to attack some rusts at all. It is possible, therefore, to interpret the results either from the viewpoint of rust susceptibility or from the viewpoint of the virulence of the isolations.

Puccinia Sorghi was found to be a common host for all of the isolations. It has been said that *Puccinia Sorghi* was most susceptible to the attacks of isolations No. 3, 5, 6, and 7, less susceptible to isolations No. 2, 3-B, and 12-2, but we can also regard isolations No. 3, 5, 6, and 7 as being more virulent than isolations No. 2, 3-B, and 12-2 on this particular species of rust.

It is interesting to note that although isolations No. 3, and 3-B were made from the same species of rust (*Uromyces Silphii*) on the same phanerogamic host (*Juncus tenuis*), but from different localities, they proved widely different in their behavior on the nineteen rusts. Since such a difference in the virulence of isolations from the same rust species, but from widely different localities, has been shown, it would appear that there are probably unlimited possibilities for strain differentiation (biological specialization). The author (Keener, 1933) has already shown differences

in the growth of these two isolations on artificial laboratory media.

Infections were secured several times on *Puccinia Anemones-virginianae* on *Anemone virginiana*, on *Puccinia Circaeae* on *Circaea alpina* (Pl. 25, fig. 8), and on *Puccinia Malvacearum* on *Althaea rosea*. It is believed that this is the first reported occurrence of *D. filum* on these rust species. In addition, the author has collections of *D. filum* on the two micro-cyclic rusts, *Puccinia curtipes* on *Micranthes virginicensis*, and on *Puccinia Xanthii* on *Xanthium pennsylvanicum*.

The eleven isolations varied considerably as to their ability to attack the three micro-cyclic rust species. The isolations also showed marked variability in their relative virulence on these hosts. Isolations No. 1, 3, 4, 5, 8, and 11-1 successfully attacked both *Puccinia Anemones-virginianae* and *Puccinia Circaeae*. Isolation No. 8 also attacked *Puccinia Malvacearum*, being the only one of the eleven isolations capable of attacking all three micro-cyclic rusts used in this work. Isolation No. 2 attacked only *Puccinia Anemones-virginianae*. This strain was not inoculated onto *Puccinia Malvacearum* and lacked the ability to attack *Puccinia Circaeae*. Isolations No. 3-B and 12-2 infected *Puccinia Circaeae* but did not attack *Puccinia Anemones-virginianae*. Both isolations No. 6 and 7 lacked the ability to attack *Puccinia Anemones-virginianae* and *Puccinia Circaeae*. In addition, isolation No. 7 did not attack *Puccinia Malvacearum*. The last mentioned isolation was the only one of the eleven that lacked the ability to attack successfully at least one of the three micro-cyclic rusts. There is thus evidence that the isolations show considerable specialization on the micro-cyclic as well as the macro-cyclic rusts.

No correlations were found between the rust genera from which the isolations were made and the genera which they were found capable of attacking. That is, an isolation from a species of the rust genus *Puccinia* showed no particular tendency to attack only species of that one genus, but was also capable of attacking species of the other rust genera used. Isolations from macro-cyclic rusts attacked both micro- and macro-cyclic forms equally well. Since no isolations were made from micro-cyclic rusts, the reverse of this situation was not determined.

In the case of negative inoculations, the results may have been due to (1) the resistance of the rust, (2) a lack of virulence on the part of the isolation, (3) to a combination of both (1) and (2), or (4) to a combination of other factors. No doubt many factors entered into the results of the inoculations. The age of the rust sorus appears to be important in determining the susceptibility of a rust. In the case of macro-cyclic species it was noted that both the youngest and the oldest uredinia were free from infections, while those sori which appeared to be in a mid-developmental stage were heavily infested. It appears that the latter sori are the most

susceptible to attack. This condition, although much more difficult to determine, seemed to prevail in field collections of *D. filum* on such rusts. For example, on *Puccinia obscura*, a rust which is commonly heavily infested with the parasite, the oldest, rusted leaves never showed fresh *Darluka* infections. The parasite, when present on leaves in such a condition, has probably invaded the rust before the leaves have died off. In the greenhouse, rusts on dead leaves of both corn and timothy could not be infected when sprayed with conidial suspensions from isolations No. 1 and 8. In all of the rust inoculations, the dried, older sori were never attacked by the parasite. This is probably due primarily to the condition of the rust itself, and not to the lack of virulence on the part of the isolation.

Another possible limiting factor to infection, is the type of sorus produced by certain species of rusts. *Puccinia poculiformis* (2) on *Poa compressa*, produces a more or less compact, cushion-shaped sorus. As has already been pointed out this rust was not attacked by any of the isolations. It is possible that this type of sorus is so constructed as to preclude any possibility of invasion. The caeoma type sorus, as produced by *Coleosporium Campanulae* and *Coleosporium Solidaginis* appears to be comparatively resistant to parasitic attack at all stages of development. Although infections of this sorus type were secured on *Coleosporium Solidaginis* in greenhouse inoculations with isolations No. 3 and 6, and although field collections of the parasite on both *Coleosporium delicatulum* on *Euthamia tenuifolia* and on *Coleosporium Solidaginis* on *Solidago* sp., were made, the numerous negative trials on the latter rust species would seem to indicate that this type of sorus is comparatively resistant. *Kuehneola Uredinis* also proved somewhat resistant to invasion by *D. filum*. Here again, it appears that the type of sorus is the factor governing the susceptibility to invasion. While it is the writer's opinion that the type of sorus in these rust species, is to a large extent responsible for the results noted, there is little actual proof of this. It may well be that many other factors are involved.

The method of inoculation may also be a factor in determining rust susceptibility. It seems possible that inoculations on such rust species as *Puccinia poculiformis* (2) on which ordinary methods failed to give positive results, might be made successful by the use of the hypodermic syringe method. Time did not permit the carrying out of this method on this particular species of rust, but if the type of sorus is such that it serves as a protection against infections from outside sources, and if the parasite can subsist on the rust hyphae as well as on the rust spores, then it might be possible to infect such a rust by the introduction of *Darluka* conidia into the host plant directly. Under any conditions, the success of the hypoder-

mic syringe method no doubt depends to a large extent on the type and texture of the phanerogamic host. For instance, an inoculation into a hard, woody plant would no doubt result only in damage to the needle. The author suggests that in future work on biological specialization among the parasitic fungi, this method of inoculation should be investigated further.

Fluctuations in temperature had some effect on the amount of infection secured. However, at no time throughout the entire course of the inoculation work, did any strain giving positive results on a particular species of rust at one temperature, fail to give the same result on the same rust species, at another temperature. Dates of inoculations were recorded, and in general it may be said that the best and most uniform infections were secured during periods of comparatively low greenhouse temperatures.

An indirect factor governing the susceptibility of certain rusts may be the general condition of the phanerogamic host as expressed by the condition of the rust. Not only was the completion of successful inoculations more difficult, but also isolations of the parasite from field materials were harder to obtain, when the phanerogamic hosts were in a semi-wilted condition. The condition of the host plant in such a situation no doubt affects the viability of both the rust and the *Darluca*.

The pycnidia of the parasite are very often embedded in the spore mass of the rust sorus and are consequently macroscopically invisible (Pl. 25, fig. 8). Such a condition was particularly noticeable in greenhouse cultures of *D. filum* on *Puccinia Anemones-virginianae*, *Puccinia Circaeae*, and *Puccinia Malvacearum* of the micro-cyclic species, and on *Puccinia poculiformis* (1), *Uromyces caryophyllinus*, and *Uromyces verruculosus* of the macro-cyclic rusts. In order to avoid erroneously reporting a negative result in such cases, loose spores were scraped from the rust sori before examining the material under the binoculars. In this manner the pycnidia were made at least partially visible. The occurrence of embedded pycnidia may possibly explain the lack of reports of *D. filum* on certain rust hosts.

Biological Specialization. Arthur (1929) has stated "Apparently some species of rusts are more susceptible to parasitic attack than others. *Uromyces Junci* and other species on *Juncus* are especially invaded and often to such an extent that the formation of teliospores is much restricted, and the same is true of some species of *Puccinia* on *Carex*." Whether or not the author had biological specialization in mind when this statement was written, is questionable. However, the results of inoculations herein reported, with various isolations of the rust parasite, *Darluca filum*, show that in reality some rusts are more susceptible than others.

The author believes that the results of his inoculations show that *D. filum* consists quite definitely of many different strains. It seems evident

that since the eleven isolations all proved distinct in their choice of rust hosts, so far as this work is concerned, we can discern eleven strains of the parasite.

The degree of specialization is not as great as in the case of the natural *Darluca* hosts—the rust fungi. Many species of rusts show a comparatively limited choice of their phanerogamic hosts. Such was not the case with the isolations of the parasite. While the eleven isolations did prove more or less dissimilar when compared with each other, any particular strain showed a comparatively wide range of potential rust hosts.

Biological specialization is of widespread occurrence throughout both animal and plant kingdoms and it is well-known among many of the fungi. The rusts are recognized as one of the most highly specialized groups of parasitic fungi and there is a high degree of differentiation within their genera and species. Up to this time, the rusts have never been grown on synthetic laboratory culture media. In spite of the apparent constant association of *D. filum* with rusts, it seems quite evident that this parasite is not as highly developed parasitically as are the rusts themselves. The culturing in the laboratory of isolations of *D. filum*, and the tendency among them to infect more than a single rust host, seems to warrant such an assumption.

It has been previously pointed out that all of the isolations attacked *Puccinia Sorghi*. Perhaps after many more isolations have been made, a strain that will not invade this rust will eventually be found. It is also probable that in view of the inoculation results with isolations No. 3 and 3-B, that after further isolations have been completed from the same rust in widely-separated localities, many intergrading forms will be found to exist. If such is the case, then strain differentiations will become far more difficult. The author is of the opinion that further strain differences may be sought from two sources. Additional strains may be expected on different species of rusts, and also on any single rust species in widely separated localities.

With the exception of the production of a stroma by isolations No. 11-1, 11-2, and 12-2 both in the field and in culture, no appreciable morphological differences between the isolations, could be detected. It seems highly possible that *D. filum* is the only valid species of the genus *Darluca* and is composed of many strains differing from each other in various degrees, but not sufficiently divergent to be classed as biological forms.

The author is greatly indebted to Dr. H. W. Thurston, Jr., of the Department of Botany, The Pennsylvania State College, for his helpful advice in the carrying out of the experimental work and in the preparation of this manuscript; to Dr. L. O. Overholts and J. W. Sinden, also of the same department for their criticisms in the preparation of the manuscript; to

Dr. C. R. Orton of the University of West Virginia, Morgantown, for the collection of the material from which isolation No. 3-B was made; and to W. L. White, a graduate student in the Department of Botany, The Pennsylvania State College, for some of the rust determinations mentioned herein and the collection of the material from which isolation No. 2 was made.

SUMMARY

1. This paper reports the results of greenhouse cultures with nine conidial isolations of *Darluca filum*. Inoculations with two isolations (one from conidia and one from ascospores) of a fungus found on *Puccinia obscura* on *Juncoides campestre* and on *Puccinia Peckii* on *Carex normalis*, in Pennsylvania, are also recorded. (See footnote, p. 477.) The latter fungus was found to be producing pycnidia and conidia of the *D. filum* type, and in addition, perithecia, asci, and ascospores, resembling those of *Eudarluca australis* Speg.

2. A total of 175 greenhouse inoculations onto 16 macro-cyclic and 3 micro-cyclic rusts were made with eleven of the thirteen isolations. Of these 101 proved to be positive while 74 gave negative results. Of the 175 inoculations, 149 were made on the 16 macro-cyclic rusts, of which 85 were positive and 64 negative. Of 26 inoculations on the 3 micro-cyclic rusts, 16 were positive and 10 gave negative results.

3. The specialization shown is interpreted in two ways: first, from the point of view of the relative susceptibility of the rusts, and second, from the point of view of the relative virulence of the isolations.

4. Two methods of measuring relative susceptibility and relative virulence are discussed.

5. *Puccinia Sorghi* was the only rust attacked by all of the isolations and *Puccinia poculiformis* (2) was the only rust not attacked by any of them. The other rusts showed degrees of variation between these two extremes.

6. It is the author's opinion that the results of the greenhouse inoculations give evidence of the existence of eleven distinct strains of *D. filum*.

7. The degree of specialization is probably not as great as in the case of the natural *Darluca* hosts—the rust fungi.

8. Infections were secured several times on the three micro-cyclic rusts, and it is believed that this is the first adequate account of attack of these rusts by *D. filum*.

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Explanation of plate 25

Photomicrographs of free-hand sections of *Darluka filum*. Magnification (with exception of Fig. 5) $\times 214$.

Fig. 1. Cross-section through innate pycnidia on *Puccinia Asparagi*.

Fig. 2. Superficial pycnidia on *Puccinia Sorghi*. Section of material from a positive inoculation.

Fig. 3. Cross-section through an erumpent pycnidium on *Uromyces Silphii* on *Juncus tenuis*.

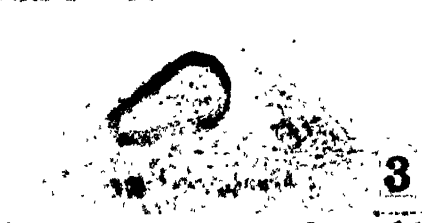
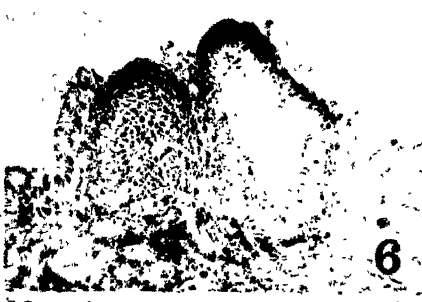
Fig. 4. Superficial pycnidia, showing fusion at the bases on *Uromyces Silphii* on *Juncus tenuis*.

Fig. 5. Cross-section through a pycnidium showing the production of conidia from the pycnidial wall cells. Material on *Puccinia obscura* on *Juncoides campestris*; $\times 445$.

Fig. 6. Cross-section through pycnidia on *Puccinia epiphylla* on *Poa pratensis*. Northern collection (Keener, No. 112432-D).

Fig. 7. Cross-section through pycnidia on *Puccinia tubulosa* on *Paspalum Humboldtianum*. Collection from Venezuela, South America (Chardon, Toro, and Alamo, No. 345).

Fig. 8. Cross-section showing pycnidia embedded in spore mass of *Puccinia Circaeae* on *Circaea alpina*. Material from a positive inoculation.



A study of pollen-tube behavior in *Lilium regale* Wil.

ESTELLA HUMPHREY

(WITH TWO TEXT-FIGURES)

Lilium regale Wil. was first introduced into this country from Western China by E. H. Wilson of the Arnold Arboretum. It is a perennial which can be propagated by crown-branching, by rhizomes, or by seeds. Seeds ripen normally in the climate of New England (Wilson, 1925)—a statement that can be made for no other species of its class; and a filial generation will mature within two years.

Although the flowers of all lilies provide excellent material for studies on pollen-tube growth, those of *Lilium regale* are particularly favorable to such work, owing to the great size of the pistil and the prolific flowering habit. The subulate, often slightly curved, style expands into a capitate three-lobed stigma, the surface of which is covered with large papillate cells. The center of the style is characterized by an open three-angled canal extending throughout its entire length. After germination, the pollen tubes push between the cells of the stigma to the stylar canal, down the inner surface of which they proceed unhindered by obstructing tissue. The total length of the style to the uppermost ovule may reach 100 mm. As many as 27 flowers have been noted on a single stem under cultivation, although plants growing in the wild seldom produce more than one-fourth this number.

The biology of reproduction in this species has been studied only by Stout (1922) at the New York Botanical Garden. He found that the plants were preponderantly self-incompatible. Of the ten plants tested, nine were self-incompatible, while one was self-compatible. Cross pollinations sometimes succeeded and sometimes failed.

The plants used in the present study were raised from bulbs obtained from a local seed house. These bulbs appear to have been derived from seed borne on plants chosen for their self-incompatibility.

Preliminary test pollinations on flowers growing in the open showed varying results due to the changes in temperature. Pollen-tube growth was slower under cool cloudy conditions than on warm sunny days, and slower during a cool night than during the day. Consequently the studies were carried on in a constant temperature chamber.

Care was necessarily taken in the emasculation process, not to tear or to spread the young undeveloped flower parts. Careless handling resulted in an apparent retardation of growth on the part of both the pistil and the pollen tubes. Emasculation made too far in advance of the dehiscence of the anthers gave the same retarding effects. For this reason it was neces-

sary to emasculate the flowers just prior to the dehiscence of the anthers. The question arises as to the actual cause of this retardation of the development of the pistil. It seems probable that it is not caused by the disturbance to the flower as a whole, but rather that, through the removal of the anthers, some part is taken away which is important for normal development of the pistil.

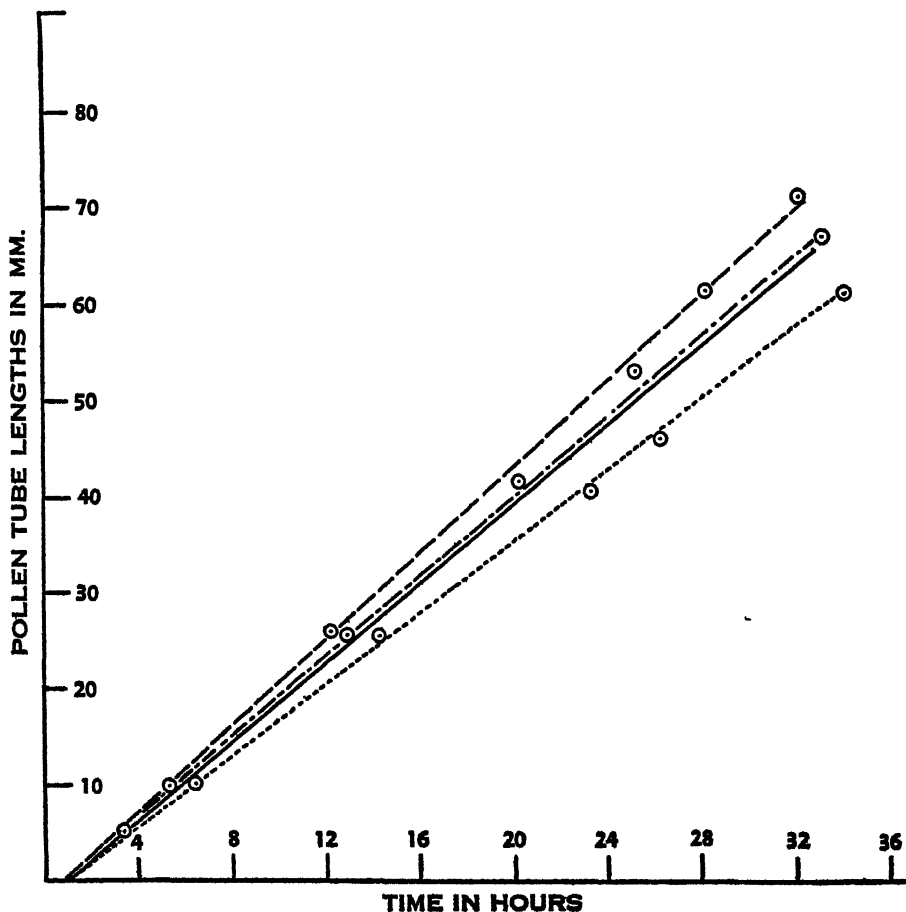


Fig. 1. Pollen-tube growth at 25°C. in cross-pollinations of *L. regale*. The solid line indicates the average growth of the three crosses.

As a rule, ten flowers of a single clon were picked, pollinated, and placed in a chamber where the humidity was uniform and the temperature was held continuously at 25°C. This manipulation was done at 9:00 A.M., since at this time the pistils were receptive to fertilization. The pollen was applied with camel's hair brushes to insure an even distribution on the stigma.

Pollen-tube growth in the pistils was studied every 6–8 hours over a period of 48 hours, or until fertilization occurred. A method of direct dissection and staining was employed. To facilitate handling in dissection, the styles—usually 80 mm. in length—were cut into 10 mm. sections. Each piece was then split longitudinally, spread flat on a slide, and stained with a few drops of aceto-carmine (saturated solution in 45% acetic acid. See Chandler, 1931). A second slide was pressed down upon the section, and the preparation was complete. When examined under the low power of a microscope, the extent of pollen-tube growth was readily determined.

Individual plants, or possibly clons, of *Lilium regale* differed in the rate of pollen-tube growth when selfed. Most of the plants were self-fertile, setting full capsules. Plant No. 5 selfed, however, showed a tendency toward self-sterility both in the pollen-tube growth and in the number of seeds set (East and Park, 1918). Figure 2 represents the growth curve of pollen tubes in this plant and also that of a selfed self-fertile plant. The latter is the only case found where there appeared to be a tendency for accelerated pollen-tube growth. Controlled selfings on the self-sterile plant produced seed capsules one fourth to one third normal size.

Cross pollinations gave generally similar results, as is seen in text-figure 1. Length plotted against time gives a straight line in which $x = \frac{1}{2}y$; or, in simpler terms, the tubes grew 2 mm. an hour. Only slight variations occur among individual plants. In figure 2, the solid line shows the average of the three broken lines in the figure. The closeness of the fit to a straight line in each case is readily apparent. In the case of delayed pollination (3:00 P.M. instead of 9:00 A.M.), the pollen tubes grew somewhat more slowly during the first 8 hours and then continued at a more rapid and normal rate until they entered the ovary.

The normal time of fertilization in selfed plants of *L. regale* is 36–40 hours after pollination. Should the growth of the pollen tubes be slowed down for any reason so that they do not reach the ovary in the time specified, the flower wilts, and fertilization fails. It follows that some pollinations may succeed and some fail on any given plant. As indicated by the curves in text-figure 2, the pollen-tube growth in the self fertile plants progressed at a constant rate. In the self-sterile plant, however, pollen-tube growth was much retarded. This is clearly indicated by the curve. After 34 hours the pollen tubes had progressed only 30–34 mm., or less than half the length of the style. At this rate, the tubes could never reach the ovary before the flower had wilted.

East (1934a, b) has shown that there are two major types of reaction affecting pollen-tube growth in the self-sterile plants of *Nicotiana* which he studied. The first is a simple nutritive reaction which produces a constant

growth-rate. The second is a reaction resembling that found in certain immunological processes. In this type a definite retardation occurs in incompatible matings due to mutual reactions between substances produced by the stigma and substances produced by the pollen tube. The acceleration which appears when compatible matings are made is apparently the reverse of the same reaction. *Lilium regale* shows a similar behavior. In addition, it is shown here that cross-pollinations as well as self-pollinations

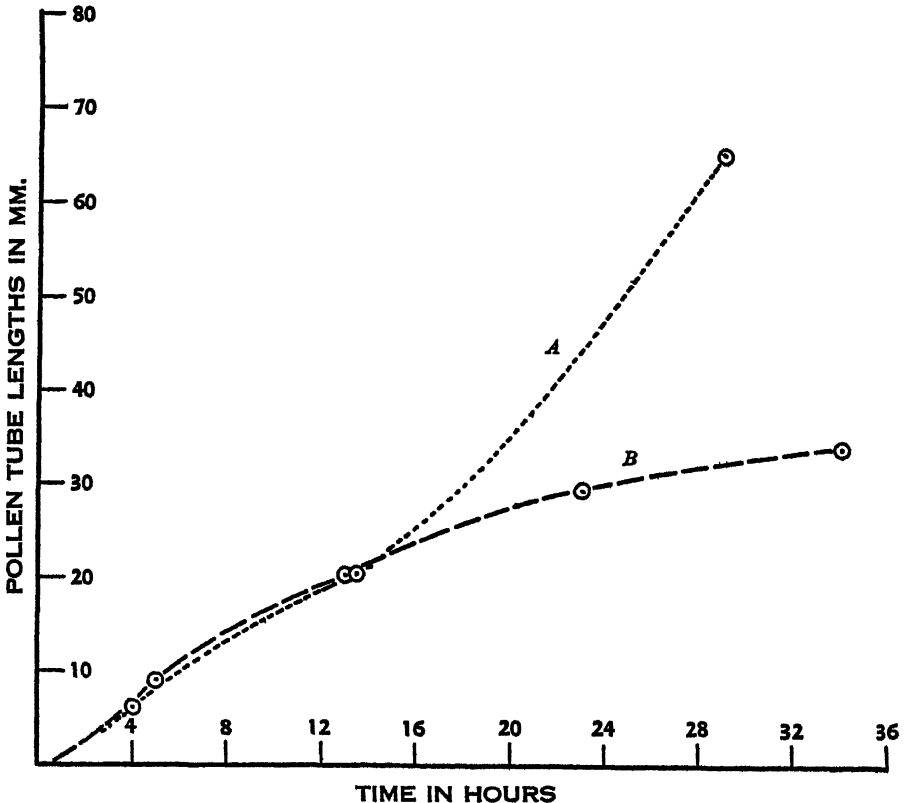


Fig. 2. Pollen-tube growth at 25°C. in two selfed plants of *L. regale*. A is self-sterile.

may exhibit the simple nutritive reaction which leads to constant growth of the pollen tubes. It is an interesting situation rather different from that ordinarily found in growth studies where there is an accumulation of adverse factors which gradually bring growth to an end. Of course it should be noted that the earliest portions of the straight-line curves must, in reality, show acceleration; but growth is constant from a period of about four hours after pollination until fertilization.

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Identification, by leaf structure, of the species of *Abies* cultivated in the United States

EDMUND H. FULLING

(WITH PLATES 26-32)

INTRODUCTION

The work reported in this paper was undertaken with a fourfold purpose:

1. To determine whether or not anatomical specificity exists, to any degree whatsoever, within the structure of the leaves of various kinds of fir.

2. If at least some such specificity be indicated to determine to what extent, if any, structural variation might preclude taxonomic use of foliar anatomy among these plants.

3. If sufficient specificity without invalidating variability be found, to prepare a key to the species of fir cultivated in the United States, the key to be based, primarily, upon leaf anatomy and supplemented only where unavoidable by external morphological characters of the leaves.

4. To prepare, in any event, a series of photomicrographs of cross sections of as many specific kinds of fir leaves as can be authentically identified representing the species cultivated in this country.

Concerning the first of these objectives, an examination of the literature upon the subject as well as the original work presented in this paper furnish an unequivocal affirmative answer. Concerning the second, differences of opinion have been expressed by previous workers and within the present paper is an evaluation of their findings in the light of the work herein reported. The third and fourth objectives constitute the principal part of this paper and merit no further comment here.

GENERAL DISCUSSION OF THE ANATOMY OF FIR LEAVES

The tissues of fir leaves, members of the genus *Abies*, may conveniently be considered under three different categories.

First, dermal tissues bound the leaf and in all cases consist of at least the epidermis, a single peripheral layer of cells, continuous except for stomatal openings. Externally, this layer is overlaid by a cuticular secretion which contributes to the general xeromorphic nature of the leaves by virtue of its universal presence, relative thickness and surprising toughness; the last-mentioned quality is especially noticeable in the difficulties inherent in paraffin sectioning of the mature organs. The stomatal apertures, according to the species, occur either only on the lower or on both surfaces

of the leaves. In either case, they occur in closely set parallel rows which on the lower surfaces generally appear as two whitish bands, one on each side of the midrib. In some, but not all, species there is next to the epidermis and constituting part of the dermal tissues, another layer or two of cells known as the hypoderm. The cell walls of this layer are generally thickened, in some species very conspicuously so, and through these walls radiating canals may usually be observed. When present and according to the species this layer is either continuous all around the leaf except directly beneath the stomata or it is more or less discontinuous because of interruptions by protrusions of mesophyll cells. When the layer is discontinuous the cells that do occur usually lie along the lateral margins of the leaf and in the center along both sides; also frequently elsewhere just within the epidermis. Regardless of the degree of continuity of this layer, it is either single-celled in thickness or two or three cells thick; the greater thickness, when present, is particularly well developed along the lateral margins and in the center along both sides. Physiologically, the hypoderm is the sclerenchymatous layer, providing partial protection against injury and extreme dessication. The epidermis shares in these functions.

Secondly, within the hypoderm is located the bulk of the leaf, the mesophyll tissue. Its constituent cells are relatively large and thin-walled as compared with those of the dermal tissues and are abundantly supplied with chloroplasts, for in these cells lies the power and function of photosynthesis. Physiologically, they constitute the chlorenchyma. This mesophyll chlorenchyma generally exhibits the two categories of palisade and spongy tissues; along the upper surfaces of the leaves and extending to the lateral margins the mesophyll cells are elongated and in vertical alignment for one or two rows; elsewhere, they are more rounded and irregularly disposed. The latter, constituting the spongy mesophyll, enclose many regularly arranged intercellular spaces which in cross sections appear as masses of torn tissue. In longitudinal sections, however, the spaces clearly alternate with strands of mesophyll. Within the mesophyll and to each side of the center is a resin canal; two canals per leaf are strikingly constant, more or fewer being anomalous though one species, *A. firma*, frequently exhibits four. The canals vary in diameter and location, according to the species, lying either against the dermal tissues, when they are known as marginal, or more or less within the chlorenchyma, being then designated as median.

Thirdly, through the center of the leaf and surrounded by the chlorenchyma extend the vascular and associated tissues which constitute the vein. They are separated from the mesophyll proper by a more or less distinct circular layer of cells, the endodermis, whose function in plants has long been a subject of much discussion. Within the endodermis, but not

occupying the entire space, are two strands of vascular tissue, more or less separate, according to the species, and entirely coalesced into one only in *Abies nobilis*; *Abies magnifica* very closely approaches the same condition. Each such strand consists of xylem cells on the upper half and phloem on the lower. Surrounding them, merging with them and filling the remainder of the space within the endodermis are the larger irregularly shaped cells of the pericyclic region. Within this region there occur, in some species, more or less distinct and sometimes very abundant and conspicuous thick-walled cells. Only in *A. firma* do such cells occasionally occur also in the chlorenchyma, at least sufficiently frequent to be of diagnostic value.

In brief, the contrasting features which appear sufficiently marked within the anatomical composition of fir leaves to be of taxonomic value as determined in this work and upon which the proposed key is based are:

1. Presence or absence of stomata on all surfaces of the leaves.
2. Presence or absence of hypodermal cells.
3. Continuous or discontinuous nature of hypoderm.
4. Moderate or excessive thickness of hypodermal cell walls.
5. One, two or more layers of cells in hypoderm.
6. Marginal, sub-marginal or median position of resin canals.
7. Size of resin canals.
8. Fused, slightly separated or distinctly separated nature of the two vascular strands.
9. Presence or absence of thick-walled cells within the pericyclic region.
10. Moderate or striking occurrence of these cells.
11. Presence or absence of thick-walled cells in the chlorenchyma.
12. Number of lines of stomata per band on leaves.
13. Thickness of epidermal cell walls.
14. Contrasts in shape of cross sections.

REVIEW OF THE LITERATURE

Thomas (1865) appears to have been the first to attribute taxonomic importance to the anatomy of coniferous leaves. He published a general discussion of the tissues involved describing, in particular, the epidermis, hypoderm, parenchyma, vascular bundle and resin canals. In addition, he discussed the more particular anatomy to be found in the leaves of the Cupressineae, Abietineae, Araucarineae, Podocarpineae and Taxineae.

Bertrand (1871) went a step further and published, not only a short general as well as a specific discussion of leaf anatomy among the firs, but

formulated a key to eighteen species based wholly upon foliar morphology. Three years later the same author presented a larger treatment of the subject covering the Gnetales and Ginkgoales as well as some twenty-five genera of the Coniferales. In it appeared a revision of his earlier key to the firs. This first attempt to distinguish species of conifers by their leaf structures contained several errors and, as Vigué and Gaussen (1929) remark, it is more of historical interest than scientific importance.

McNab (1875–1877), in a series of papers, gave a revision of the genus *Abies* and discussed the foliar anatomy of several particular species.

Engelmann (1878) appears to have been the first to consider, in particular, the foliar anatomy of the American firs. He prepared a rather detailed study of them including an anatomical key.

Fedtschenko (1879), apparently having implicit faith in the taxonomic value of leaf anatomy, founded a new species, *A. Semenovii*, upon such criteria. Beissner (1898), however, criticized this work and Fedtschenko's species has since been disregarded.

Medwedew (1880) studied only *A. Nordmanniana*. Meyer (1883) was impressed by the taxonomic significance of the resin canals in the leaves. Masters (1889 and 1891) made further general and specific observations. Trabut (1889) studied four Mediterranean species and published very fine plates of their foliar anatomy. Some years later (1906 and 1928) he, like Fedtschenko, appears to have established a new species, *A. Marocana*, partly at least upon leaf structure.

After Engelmann, Lemmon (1889–1890) was the next American writer to discuss leaf anatomy, which he did in connection with a very excellent report upon all the conifers of California.

Daguillon (1890), in a series of articles, furnished considerable data upon the subject. The following year Van Tieghem (1891) did likewise and, in addition, formulated an anatomical key to twenty-five species.

Sargent (1892), in his classic "Silva," was the third important American contributor. Bastin and Trimble (1896) next took up the American side of the investigations and in a series of articles published data and pictures of fir-leaf anatomy. Hickel (1906 and 1908) made extensive studies of the genera in the Abietineae and formulated keys to the species, based partly upon leaf anatomy. Guinier and Maire (1908) made various allusions to the leaf anatomy of the Mediterranean firs. Lamb (1912–1914) did likewise in the case of the American species. The extensive bulletin by Zon (1914) upon the balsam fir naturally also contained anatomical data. Bode (1914) gave some consideration to an unimportant species, *A. Nebrodensis*. Sharp (1915), who published anatomical studies of all the native genera, appears to have been the fifth to give serious attention to the American conifers.

The paper by Durrell (1916) is valuable because of its drawings of every species. Sudworth (1916) continued these considerations with his dendrological bulletin. Pujuila (1921) studied some varieties of the Spanish fir. Mattfeld (1925) published a valuable paper with anatomical, distributional and other considerations of the Mediterranean firs. In Japan two studies upon the subject in hand have been made, those of Ohki (1925) and of Hayata and Satake (1929). The establishment of the two latest species to be added to the genus *Abies* was based partly upon leaf anatomy by Gaussen (1928) and by Flous and Gaussen (1932).

Finally, the most extensive morphological study of the firs is the revision of the genus by Viguié and Gaussen (1928 and 1929). In this monographic work 52 species and 12 varieties of fir are morphologically described with respect to leaves, branches, buds, flowers and cones. For some reason, a bibliography very unfortunately does not accompany this paper though an extensive review of literature is included.

Certain histological regions within the leaves of firs have received special attention in attempts, not only to describe them anatomically but, more important, to discover their functions and phylogenetic importance. Soar (1922) published a worthy contribution in this respect upon the endodermis in the leaves of the Abietineae. Among her observations the one most pertinent to the problem in hand probably is that hypodermal tissues in the leaves of *Picea* and *Abies* are most strongly developed near the bases where there is incomplete development of the endodermal sheath. This fact emphasizes the necessity, for taxonomic purposes, of considering foliar cross sections from the middle of the leaf and not near the base.

Within the endodermis, as already indicated, is located the vascular tissue consisting of both xylem and phloem cells surrounded by the larger and looser cells of the pericyclic region. In gymnospermous leaves varying amounts of this region are occupied by short parenchymatous tracheids possessing bordered pits. Such cells constitute the so-called "transfusion tissue" which, according to Haberlandt, is poorly developed in *Abies* but greatly in *Pinus*. The interpretation, functionally and phylogenetically, of this transfusion tissue has engaged several investigators. Frank (1864) appears to have first observed it in *Taxus baccata* where he regarded it as arising from the bundle proper.

From that time until 1913 a number of contributions with conflicting ideas were published by Thomas, von Mohl, Bertrand, de Bary, Zimmerman, Scheit, Vettters, Daguilleon, Van Tieghem, Lignier, Worsdell, Bernard, Chauveaud, Carter, and Takeda. In brief, their ideas represent two schools of thought, the one regarding transfusion tissue as centripetal xylem and a part of the vascular bundle itself, the other looking upon it as pericyclic

or parenchymatous in origin. Concerning the function of the tissue some have held it to be for storage, others as an auxiliary conducting system.

Chauveaud (1904), concerning himself with the double nature of vascular bundles in the leaves of firs and pines, decided, in the cases of *A. venusta* and *A. Pinsapo*, that the vascular strand is at first undivided and becomes divided during the course of development through secondary modifications. Hill and de Fraine (1908 and 1909), as well as Hickel (1911), studied, among other features, cotyledons of the firs. Gyorffy, in a number of articles, was interested in the occurrence and morphology of double needles. Stomata occupied the attention of Wilhelm (1883), Hildebrand (1860) and Mahlert (1885). F. Darwin (1887) was concerned about the relation between "bloom" and stomata. Florin (1932) discusses stomata in his recent paleobotanical studies.

The influence of environmental conditions upon the anatomy of fir leaves has not been overlooked. Hartmann (1892) and, to a more informative degree, Anderson (1897), were concerned with abnormal foliar anatomy resulting from pathogenic infection. Climatic influences upon foliar anatomy engaged the attention of Areschoug (1882) in a general way. Mer (1883) was concerned with similar influences of light and shade upon the leaves of Norway spruce. Noack (1888) was interested in the influence of climate upon cuticularization and Hessmer (1916) studied the differences between exposed and shaded leaves of evergreens. Taubert (1926) pursued similar investigations upon the leaves of *Abies* in one of the best contributions upon the subject. Liese (1929) and Mussiuk (1932) observed similar differences in the Scotch pine.

Dependability of anatomical features for taxonomic purposes has been implied if not directly defended in every work proposing keys. It is obvious that to those sharing this viewpoint structural constancy of foliar organs appears greater than any variability that may at the same time occur in them. Other observers, however, impressed more by exceptions to the rule regard foliar structure as unreliable for purposes of determination. Von Wettstein (1887), apparently writing of Austrian plants, said the coniferous species of his flora could be more accurately determined by foliar anatomy and that such examination presents the surest means of recognizing hybrid forms. Masters (1891) admitted that some anatomical features possess diagnostic value to a certain degree. Petunnikov (1900), however, impressed by the work of Duval-Jouve (1875) upon the grasses and by that of Bertrand (1874), Koehne (1893) and Fedtschenko (1898) upon *Abies*, denies any such value. Fritsch (1903) discusses this matter in a general sense and makes the following statement which expresses the viewpoint adopted by the present writer in undertaking the work in hand: "In

no case can one depend on anatomy alone in generic and specific distinction, but if we take it hand in hand with the external characters we shall very frequently find that the two supplement one another in a most agreeable manner." Hamilton (1916) wrote about the instability of leaf morphology in its relation to taxonomic botany. Maleev (1929) expresses other views.

Gauba (1927) writes of "Metakutisierung" of resin canals, probably meaning cuticularization of their walls and says that examination of 27 coniferous genera with over 100 species indicates that this feature possesses no systematic value.

Several other genera of plants have also been investigated with reference to their foliar anatomy. Among the angiosperms only the early and recent very extensive studies of Duval-Jouve (1875) and Prat (1932) upon the grasses can be mentioned. Every family of the gymnosperms appears to have received attention, and of the genera most closely related to *Abies* the following studies deserve recognition.

Pinus: McNab (1875), Purkyne (1875), Menge (1878), Engelmann (1880), Mahlert (1885), Coulter and Rose (1886), Van Tieghem (1891), Bastin and Trimble (1897), Masters (1904), Pardé (1912), Shaw (1914), Doi and Morikawa (1929), Hayata and Satake (1929), Grigorieva (1930), Harlow (1931), Fieschi and Gaussen (1932), Fieschi (1932), and Sutherland (1934). Among these, the works of Harlow and of Sutherland are most worthy of consideration because of their specific descriptions, anatomical keys and photomicrographs. The so-called pines of Australia, which include a number of southern hemisphere gymnosperms, none of which belong to the genus *Pinus*, are extensively treated by Baker and Smith (1910).

Picea: Brunet (1866), Bastin and Trimble (1897), Hayata and Satake (1929), Gaussen and Lacassagne (1930) and Marco (1931), *Tsuga*: McNab (1876), Bastin and Trimble (1897). *Pseudotsuga*: McNab (1876), Koch (1877), Flous and Gaussen (1932). *Taxodium*: Coulter (1889). *Cupressus*: Maxwell (1896), Camus (1914). *Torreya*: Hayata and Satake (1929). *Sciadopitys*: Hayata and Satake (1929). *Juniperus*: Hayata and Satake (1929).

In addition to all the above-mentioned more or less special contributions upon the subject in hand, one finds abundant taxonomic reference to foliar anatomy of firs in many general treatises. Among these are those of Carrière (1855 and 1867), Mayr (1890), Beissner (1891, 1899, 1909, 1930), Koehne (1894), Sargent (1898, 1905 and 1916), Elwes and Henry (1909), Pardé (1913), Clinton-Baker (1913), Wilson (1916), Coltman-Rogers (1920), Dallimore and Jackson (1923), Bailey (1923), Engler and Prantl

(1926), Rehder (1927), Fitzpatrick (1929) and Mattfeld (1928 and 1930).

Numerous general remarks, not especially taxonomic but otherwise informative, have come to the writer's attention in the following references: Goeppert (1841), DuHamel (1777), Zuccarini (1843), Schacht (1853), Hempel and Wilhelm (1889), Masters (1880 and 1891), Dammer (1900), de Bary (1884), Feustel (1921).

PROCEDURE

Sources and selection of material

To determine what species of fir are in cultivation in this country Rehder's list was originally accepted. In the recent edition of Bailey's manual (1933), which appeared during the course of this investigation, one more species, *A. religiosa*, is described which was not included in earlier editions of either work. Of the 38 species thus listed as being cultivated, some are not yet extensively grown. These, however, have been included in the present study because of the prospects of their increased use in horticulture. Varieties have been omitted principally because of their relative unimportance and the added complications their inclusion might have caused.

In the belief that fresh living leaves would offer more natural objects for study, efforts were made to secure, so far as possible, such material. Local pineta presented the most immediate sources in this respect. From the following collections, accordingly, living material of almost every species reported to be in cultivation was secured:

Arnold Arboretum, Jamaica Plain, Mass.
Pinetum of the New York Botanical Garden.
Pinetum of Childs Frick, Roslyn, N.Y.
Pinetum of T. A. Havemeyer, Brookville, N.Y.
Pinetum of R. H. Montgomery, Cos-Cob, Conn.
Pinetum of A. G. Hodenpyle, Locust Valley, N.Y.
Pinetum of W. R. Coe, Oyster Bay, N.Y.
Pinetum of George Brett, Fairfield, Conn.

In addition, requests for material were sent to forest supervisors and other persons in similar positions scattered throughout the western states. By their kind assistance fresh specimens of our native species, packed in damp moss or other suitable moist substance, were thus secured. The letters sent to these various persons, in addition to stating the nature and purpose of the request, were accompanied by mimeographed sheets to be filled in by the collector for the purpose of furnishing information concerning the geographical and altitudinal locations of the trees sampled and

whether each twig sent was secured from the lower sunny, lower shaded, upper sunny, or upper shaded side of the tree. These four classes of twigs from each tree were especially requested because the writer wished to determine, so far as was practically possible, if there might be sufficient anatomical variation within these parts of the tree to discredit any other-wise apparently valid distinguishing features. The cooperation on the part of the persons solicited was especially gratifying, furnishing the following material:

<i>Species</i>	<i>Elevation</i>	<i>Location</i>
<i>arizonica</i>	9000'	Beaver Creek Reservation, Rio Grande National Forest, Arizona.
<i>amabilis</i>		Vancouver Is., B.C., Canada.
<i>balsamea</i>		Ottawa, Canada.
		Moore Factory, Canada.
<i>Fraseri</i>		North Carolina.
		West Virginia.
<i>grandis</i>		Vancouver Is., B.C., Canada.
		University of California campus.
		Wenatchee Mts., Washington.
<i>concolor</i>	8500'	Alamo Camp, Rio Grande National Forest, Arizona.
		Cloudcroft, New Mexico.
	8000'	Manti National Forest, Utah.
	9000'	Manti National Forest, Utah.
	7000'	Manti National Forest, Utah.
	6600'	Sequoia National Forest, Cal.
		Heber, Utah.
		Albuquerque, New Mexico.
<i>lasiocarpa</i>	7500'	Gallitin National Forest, Mont.
	8000'	Sawtooth National Forest, Idaho.
	9200'	Medicine Bow National Forest, Wy.
	8500'	Manti National Forest, Utah.
	7200'	Targhee National Forest, Idaho.
		Heber, Utah.
		Vancouver Is., Canada.
<i>magnifica</i>	7500'	Sequoia National Forest, Cal.
	7600'	Sequoia National Forest, Cal.
<i>nobilis</i>		Wenatchee Mts., Washington.
<i>venusta</i>		University of California campus.

Lastly, the writer personally secured specimens of *A. balsamea* from Livingston Manor in the Catskill Mts. of New York and from the Douglas Lake region of northern Michigan; also *A. Fraseri* from the northern limits of its range in West Virginia.

Determination of species

Inasmuch as the value of any conclusions derived in this work would be largely dependent upon the certainty of the identification of the specimens examined, great care was exercised in determining the species of material employed. By diligent consultation of standard taxonomic and dendrological works, coupled with the field work involved in collecting, the writer sufficiently familiarized himself with the kinds of fir in cultivation so that he finally felt certain of the material eventually selected for study. The principal references consulted for this purpose were those of Sargent (1898), Rehder (1927), Dallimore and Jackson (1923) and Beissner-Fitschen (1930). Every branch from which leaves had been examined was dried and mounted and compared with herbarium specimens kindly lent by the Arnold Arboretum, as well as with those in the herbarium of The New York Botanical Garden.

Technique

Celloidin Method

The following schedule of technique, adapted from Harlow with some modifications, was employed in this work:

1. Tie needles in bundles of 15 to 30 with thread a little to one side of their centers.
2. Cut off, with razor, each end of bundle leaving a fagot about $\frac{1}{4}$ to $\frac{1}{2}$ inch long.
3. Dehydrate fagot in 50% alcohol under reduced pressure of water pump until bubbles cease to rise from needles.
4. Provide at least two changes of absolute alcohol during next 48 hours.
5. Change to 50-50 mixture of absolute alcohol and ether for 12-24 hours.
6. Immerse in thin solution of celloidin dissolved in 50-50 mixture of absolute alcohol and ether, about consistency of glycerin, in small glass bottles provided with tight cork and metallic screw cap.
7. Leave bottles in paraffin oven for one week.
8. Remove from oven, allow to cool partly, open and permit celloidin to thicken slowly by evaporation of alcohol-ether. Assist by occasional stir-

ring to prevent formation of surface film as celloidin must be kept homogeneous during this process, which occupies a few hours. Thicken to a consistency obviously greater than originally, re-cork and cap and put in oven; or replace celloidin with thicker celloidin. This latter method is quicker though more troublesome because of the nature of celloidin.

9. Repeat last operation in a few days or a week until celloidin is so thick that a mass of it removed on an instrument will no longer run but congeal on exposure to the atmosphere.

10. Remove bundle with adhering celloidin by means of forceps into small amount of chloroform which solidifies the celloidin. Leave for 2 to 12 hours. Longer immersion does no harm; on the contrary, it insures more uniform solidification of the celloidin.

11. Cut on sliding microtome with oblique knife, through part of fagot corresponding to center of original needles. Lubricate blade and celloidin block with 70% alcohol for each section. Cut 24 to 30 microns thick. If sections roll on blade unroll them with brush just before completing stroke through block. Remove with brush into 70% alcohol.

Two methods of staining technique were employed. The first was:

12a. Add to 70% alcohol containing sections a few drops of 1% Bismarck brown in 70% alcohol; stain to desired intensity.

13a. Pour off Bismarck brown and wash in 70%, 95% and two washes of 100% alcohol, two to five minutes in each, half hour at least in last of absolute. To each washing of absolute add a few drops of chloroform to prevent softening and possibly dissolution of celloidin matrix. If, in spite of precautions, the matrix does soften causing sections to adhere together or loosen and thus render their subsequent individual handling difficult, the chloroform should be omitted and the celloidin allowed to dissolve in the alcohol, even with the assistance of a little ether. If so used, the ether must be removed by washings in absolute alcohol previous to the subsequent application of xylol. By this latter procedure the sections are at least rescued from a gummed mass of celloidin though their mounting without a matrix becomes more difficult.

14a. Pour off alcohol and give at least two washings of xylol, at least half an hour in last one.

15a. Mount in balsam.

For photomicrographic purposes the above as well as the following schedule, adopted from Harrar, were found satisfactory:

12b. Dilute 70% alcohol containing sections with about same amount of distilled water.

13b. Add a few drops of aqueous ferric ammonium sulfate, a mordant, and allow to remain about five minutes.

14b. Pour off, wash twice in distilled water, add some distilled water and a few drops of aqueous haematoxylin. Watch staining under microscope to desired intensity.

15b. Pour off, wash twice in distilled water, pour on 70% alcohol and add a few drops of Bismarck brown.

16. Same as in 13a to 15a.

It was found more satisfactory in some cases for photographic purposes to remove the celloidin by ether, preferably after staining, and then to select under a hand lens a few of the best sections for mounting. Most pictures were obtained, however, of sections in a celloidin matrix.

Pressure chamber and paraffin

Before the details of the above described celloidin technique were satisfactorily developed, a pressure chamber, as suggested by Lodewick, was devised. Supposedly, such an apparatus would secure more rapid penetration of the material by the celloidin. The method, however, was not satisfactory so far as it was attempted.

Though young immature coniferous needles are known to section well in paraffin, older ones, according to Chamberlain, do not lend themselves to such treatment. This was found to be true in attempts to employ paraffin in the butyl-alcohol technique. Rather than attempt modifications of this method, such as Hance suggests, this technique, too, was abandoned and all the work was performed as outlined above.

Herbarium material

In spite of efforts to secure fresh leaves a few species remained unrepresented in the writer's collections. Inasmuch as these, too, were reported to be in cultivation, though apparently not extensively so as yet, it became necessary to resort to herbarium material in order to include them. In so doing it was found that the quality of herbarium material for anatomical purposes varies considerably, probably depending upon drying methods employed in connection with such specimens. Internal structure was almost perfectly preserved in some cases; badly distorted in others. Unsatisfactory material was usually indicated by excessive shrinkage of the mesophyll along the midrib resulting in prominence of the latter. This was apparent by hand-lens inspection of leaves merely cut across. Those that appeared least distorted were run through the celloidin method with the addition of the following between items #2 and #3 of that schedule:

Boil fagots in water for 5 to 10 minutes, transferring them momentarily once or twice during the boiling into cold water to insure penetration.

RESULTS

Concerning anatomical variability

That anatomical variability might invalidate apparently acceptable diagnostic features in fir-leaf anatomy was well recognized before the work herein reported was undertaken. Previous investigators differed in their opinions upon this point. Some appear to have regarded variability as sufficient to disqualify attempts at classification by foliar anatomy; others apparently looked upon it only as exceptions to general rules. In choosing between these two viewpoints one might be inclined to be more impressed by those who found exceptions, for such results usually indicate more thorough investigation. It became necessary for the present writer, consequently, to arrive at some decision with regard to this controversial matter. To do so he hoped to be able to study material of each species from so many different locations as well as from different parts of the same trees that all possibilities of variation would come under his observation. It soon became apparent, however, that such ideal methods were beyond attainment. In compromise, he realized this objective in two native species only and then arbitrarily adopted a suggestion of Taubert.

The two native species which lent themselves to a study of variability were *A. concolor* and *A. lasiocarpa*. In addition to specimens gathered in cultivated collections others, as already noted, were secured from a number of locations within their natural ranges of distribution. Altitudinal and geographical influences, to some degree at least, were thus observable. Three- and four-year old needles from the lower sunny and shaded and the upper sunny and shaded portions of each tree were examined. In each of these groups three bundles of needles were prepared thus making 24 bundles per tree each containing at least 10 needles. From each such bundle at least 20 sections were cut. Over 4000 cross-sections of leaves were thus prepared from each tree considered. While it cannot be said that so many were always critically examined, the chances of discovering important variations were certainly greatly increased. In spite of this painstaking effort to detect structural deviations the anatomical features in these two species were so constant, at least concerning the characters necessary for identification, that in them variability was negligible. The positions of resin canals, the thickness of hypodermal cell walls, the degree of separation of vascular strands and various other details were observed to vary slightly, but no correlations with environment and positions on the tree could be made with justification.

These two species are among the most easily identified of the firs. Because of this the writer would scarcely have been justified in assuming that

Summary of anatomical characters in the leaves of cultivated species of Abies

SPECIES	STOMATA	HYPODERM	CANALS	BUNDLES	THICK-WALLED CELLS IN PERICYCLIC REGION		REMARKS
<i>A. alba</i>	On lower surface only	Discontinuous, double in places	Marginal	Separated	Few		
<i>A. amabilis</i>	On lower surface only	Continuous	Marginal	Separated	Absent		
<i>A. arizonica</i>	On both surfaces	Continuous	Median	Separated	Absent		Indistinguishable anatomically from <i>A. lasiocarpa</i> .
<i>A. balsamea</i>	On lower surface only	Discontinuous or absent	Median	Separated	Absent		Distinguishable from <i>A. Fraseri</i> only when hypodermal cells are absent.
<i>A. Beissneriana</i>	On lower surface only	Continuous, double in places	Marginal	Separated	Present		Lateral margins are bluntly pointed.
<i>A. Borisii-regis</i>	On lower surface only	Continuous, double in places	Median	Separated	Present		
<i>A. Bornmuelleriana</i>	On lower surface only	Continuous	Sub-marginal	Separated	Present		
<i>A. cephalonica</i>	On lower surface only	Continuous, double in places	Marginal, small	Separated	Present		Pointed leaves tending to be radially arranged are distinctive.
<i>A. chensiensis</i>	On lower surface only	Continuous	Marginal	Separated	Present		Bluntly pointed lateral margins of cross sections are quite distinctive.
<i>A. cilicica</i>	On lower surface only	Continuous, double in places	Marginal	Separated	Present		
<i>A. concolor</i>	On both surfaces	Discontinuous	Marginal	Separated	Rare		
<i>A. Delavayi</i>	On lower surface only	Continuous	Marginal	Separated but close	Present		Leaves are markedly revolute.
<i>A. Fargesii</i>	On lower surface only	Discontinuous	Median to sub-marginal	Separated	Present		
<i>A. Faxoniana</i>	On lower surface only	Continuous	Median	Separated	Present		
<i>A. firma</i>	On lower surface only	Continuous, very thick-walled	Median	Separated	Present		Only species with thick-walled cells in chlorenchyma.
<i>A. Forrestii</i>	On lower surface only	Discontinuous	Median	Separated	Present		Leaves are slightly revolute.
<i>A. Fraseri</i>	On lower surface only	Discontinuous	Median	Separated	Absent		Distinguishable anatomically from <i>A. balsamea</i> only when hypodermal cells are absent from latter.

<i>A. grandis</i>	On lower surface only	Absent or few cells present	Marginal	Separated	Rare	
<i>A. holophylla</i>	On lower surface only	Continuous, double in places, thick-walled	Median	Separated	Abundant	Long pointed needles on young plants are very distinctive.
<i>A. homolepis</i>	On lower surface only	Continuous	Median	Separated	Abundant	
<i>A. koreana</i>	On lower surface only	Absent, few cells may be present	Marginal-sub-marg.	Separated	Absent	
<i>A. lasiocarpa</i>	On both surfaces	Discontinuous	Median	Separated	Absent	
<i>A. magnifica</i>	On all surfaces	Discontinuous 2-4 cells thick in angles	Marginal	More or less fused	Absent	Only species with more or less four-sided leaves bearing stomata on all surfaces. Vascular strands more or less fused.
<i>A. Mariesii</i>	On lower surface only	Absent	Median-sub-marg.	Separated	Absent	
<i>A. nephrolepis</i>	On lower surface only	Discontinuous	Median	Separated	Present	
<i>A. nobilis</i>	On both surfaces	Discontinuous	Marginal	Fused	Absent	Only species with completely fused vascular strands. Marked groove along upper surfaces of leaves.
<i>A. Nordmanniana</i>	On lower surface only	Discontinuous	Marginal	Separated	Absent	
<i>A. numidica</i>	On lower surface only	Continuous, double in places	Median	Separated	Absent	Stomata sometimes on upper surface.
<i>A. Pindrow</i>	On lower surface only	Continuous	Marginal	Separated	Present	
<i>A. Pinsapo</i>	On both surfaces	Continuous or disc. Double in places	Marginal-Median	Separated	Absent	Very distinct species because of stomata on upper surfaces of leaves which radiate in all directions from twig.
<i>A. recurvata</i>	On lower surface only	Continuous, double in places	Marginal	Separated	Present	Very distinct when leaves are bent back along twig.
<i>A. religiosa</i>	On lower surface only	Continuous	Marginal	Separated	Present	
<i>A. sachalinensis</i>	On lower surface only	Absent	Median	Separated	Present	Canals large.
<i>A. sibirica</i>	On lower surface only	Absent	Median	Separated	Absent	Canals large.
<i>A. spectabilis</i>	On lower surface only	Continuous	Marginal	Separated	Present	Cross-sections sometimes bluntly pointed at lateral margins.
<i>A. squamata</i>	On lower surface only	Continuous	Median	Separated	Present	Leaves unusually thick and resin canals distinctly median.
<i>A. sutchuenensis</i>	On lower surface only	Continuous	Median	Separated	Present	Said to be distinguished by its yellow petioles.
<i>A. Veitchii</i>	On lower surface only	Absent	Median	Separated	Present	
<i>A. venusta</i>	On lower surface only	Continuous	Marginal	Separated	Absent	No groove along upper surfaces of leaves.

their anatomical constancy indicated equal freedom from variability among other species. It was impossible to investigate this matter, however. In order, then, to reconcile dissension among previous investigators Taubert's suggestion was accepted that to give value to leaf structure one must specify the part of a tree from which the leaves to be considered are secured. To fulfill this condition the writer arbitrarily based his work, so far as was possible, upon leaves secured from the lower part of a tree, preferably the shaded side, for on every plant there is one side that is at least not in direct sunlight. In the case of herbarium material such information is almost never stated, and for that reason herbarium material was used as little as possible.

It must be stated, however, that no anatomical feature can safely be determined by an examination of only one or even a few leaves. As many as is practically possible should be examined, for frequently it is only by an average of many observations that one may be able to assign a particular character to one or another category. This is especially true concerning continuity of the hypoderm and location of resin canals. In the case of *A. firma*, for example, the occurrence of thick-walled cells in the chlorenchyma is very distinctive, but they do not appear in every section. Sections examined must be from the middle part of the leaves for toward the ends the position of resin canals and abundance of hypoderm varies. Leaves three or four years old were always used.

Finally, the writer feels it necessary to state that he does not propose the key presented in this paper as a substitute for the longer established means of identification based upon cones, buds, twigs and leaves. He offers it only as a supplement to those methods, especially in cases where certain external characters cannot be observed. This is particularly true in spring and early summer before the winter buds form.

KEY TO THE CULTIVATED SPECIES OF ABIES BASED UPON STRUCTURE OF LEAVES FROM STERILE BRANCHES

A. Stomata nearly equally numerous on all surfaces.¹

B. Resin canals median.

C. Leaves usually not over 2 cm. long, radially arranged on twig. *A. Pinosapo*

CC. Leaves usually over 2 cm. long, not radially arranged on twig. *A. lasiocarpa*
*A. arisonica*²

¹ The presence or absence of stomata on all surfaces of the leaves can be determined most accurately by macroscopic examination. Species under "AA" sometimes have a few stomata on the upper surface near the tip and occasionally near the center, the latter being observable in median cross-sections. They never approach the abundance, however, of those on the under surface as in species under "A."

- BB. Resin canals marginal.
- C. Vascular bundles appearing as one or nearly so.
- D. Cross-sections rhomboidal in shape; vascular bundles sometimes slightly separated *A. magnifica*
- DD. Cross-sections not rhomboidal in shape; groove along upper surface frequently very sharp; vascular bundles always appearing as one. *A. nobilis*
- CC. Vascular bundles distinctly separated.
- D. Leaves usually not over 2 cm. long, radially arranged on twig. *A. Pinsapo*
- DD. Leaves usually over 2 cm. long, not radially arranged on twig. *A. concolor*
- AA. Stomata abundant only on lower surface.¹
- B. Hypodermal layer absent; occasional individual cells, more or less thick-walled, directly beneath epidermis, sometimes in groups, but never sufficiently abundant to constitute what might be regarded even as a discontinuous layer.
- C. Thick-walled cells present in pericyclic region though not necessarily conspicuous.
- D. Resin canals marginal. *A. grandis*
- DD. Resin canals median.
- E. Resin canals narrow, 1/4 to 1/3 as wide as leaf. *A. Veitchii*
- EE. Resin canals wide, 1/3 to 1/2 as wide as leaf. *A. sachalinensis*
- CC. Thick-walled cells absent from pericyclic region.
- D. Resin canals marginal to sub-marginal.
- E. Leaves not over 2 cm. long, pointed, rounded or emarginate at apices, revolute at margins. *A. koreana*
- EE. Leaves up to 6 cm. long, rounded or bifid at apices. *A. grandis*
- DD. Resin canals median to sub-marginal; some stomata frequently present on upper surface.
- E. Leaves distinctly slender as far as fir leaves are concerned, many not over 1 mm. wide, and up to 3 cm. long *A. sibirica*
- EE. Leaves usually at least 1.5 mm. wide and not over 2.5 cm. long.
- F. Resin canals distinctly median; some stomata frequently present on upper surface. *A. balsamea*
- FF. Resin canals more or less submarginal; stomata absent from upper surface. *A. Mariesii*
- BB. Hypodermal layer present, continuous or discontinuous, at least along the upper surface and at lateral margins, sometimes extending to lower surface but never in immediate region of stomata.
- C. Hypodermal layer more or less continuous, only an occasional cell or two lacking along upper surface.
- D. Thick-walled cells absent from pericyclic region.
- E. Leaves frequently stomatiferous on upper surface near apex. *A. numidica*
- EE. Leaves not stomatiferous on upper surface *A. amabilis*
- DD. Thick-walled cells present in pericyclic region.
- E. Leaves more or less revolute.
- F. Leaves distinctly revolute appearing ω -shaped in cross-section *A. Delavayi*
- FF. Leaves only moderately revolute. *A. Forrestii*
- EE. Leaves not revolute.
- F. Thick-walled cells frequently present in chlorenchyma, not necessarily in every section. *A. firma*

¹ This species is usually regarded as a variety of *A. lasiocarpa* from which it differs, primarily, in possessing thick, corky, creamy-white bark.

FF. Thick-walled cells absent from chlorenchyma.

G. Resin canals median or sub-marginal.

H. Hypodermal-cells conspicuously thick-walled.

I. Leaves spiny pointed.....*A. holophylla*II. Leaves blunt or bifid.....*A. homolepis*

HH. Hypodermal-cells moderately thick-walled.

I. Cross-sections showing more or less prominent broad midrib on lower surface, groove along upper surface and resin canals nearer margins than vascular bundle.....*A. Bornmülleriana*³*A. Borissi-regis*

II. Cross-sections showing no prominent broad midrib along lower surface.

J. Resin canals distinctly median, equally distant from margins and vascular bundles; leaves half as thick as wide.....*A. squamata*JJ. Resin canals median but tending to be nearer margins; leaves only one third as thick as wide and tapering toward margin....*A. Fargesii*⁴*A. Faxoniana*

GG. Resin canals marginal or sub-marginal.

H. Groove absent along upper surface resulting in straight upper margin of cross-sections; leaves spiny-pointed.....*A. venusta*

HH. More or less distinct groove present along upper surface; leaves sharp-pointed in some.

I. Lateral margins bluntly pointed.

J. Leaves up to 6 cm. long; epidermal and hypodermal cells quite similar in size and wall-thickness.....*A. spectabilis*JJ. Leaves not over 3 1/2 cm. long; hypodermal cells more conspicuous than epidermal and generally thicker-walled.....*A. chensiensis*

II. Lateral margins more or less rounded.

J. Hypodermal layer frequently 2 or 3 cells thick, especially along lateral margins and midrib.

K. Leaves sharply pointed...*A. cephalonica*KK. Leaves not sharply pointed...*A. cilicica*

JJ. Hypodermal layer usually one cell and only occasionally 2 or more cells thick.

K. Leaves sharply pointed or at least acute, bent backward along twig...*A. recurvata*

KK. Leaves usually not sharply pointed; never bent backward.

L. Leaves up to 6 cm. long.....

.....*A. Pinárow*⁵*A. spectabilis*³ These two species differ chiefly in the presence of dense short pubescence on the branchlets of *A. Borissi-regis*.⁴ Of these two species only *A. Faxoniana* has branchlets which are densely pubescent.

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Explanation of plates 26-32

Fig. 1. *A. chensiensis* Van Tiegh., Bull. Soc. Bot. France 38: 413 (1891): bluntly pointed lateral margin, marginal resin canal and moderately thick-walled hypodermal cells. China.

Fig. 2. *A. Veitchii* Lindl.: median resin canal, absence of hypodermal layer and presence of stomata only on lower surface.

Fig. 3. *A. nephrolepis* Maxim.: discontinuous hypoderm composed of moderately thick-walled cells.

Fig. 4. *A. firma* Sieb. & Zucc.: excessively thickened walls of hypodermal cells.

Fig. 5. *A. firma* Sieb. & Zucc.: two slightly separated vascular strands, excessively thick-walled cells in the pericyclic region within the endodermis and two such cells in the chlorenchyma outside the endodermis.

Fig. 6. *A. magnifica* Murr.: a region of the hypoderm composed of several rows of moderately thick-walled cells.

Fig. 7. *A. balsamea* Mill.: longitudinal section of leaf showing intercellular spaces in palisade parenchyma.

Fig. 8. *A. Nordmanniana* Spach: marginal resin canal and continuous hypoderm composed of one layer of moderately thick-walled cells.

Fig. 9. *A. nobilis* Lindl.: unseparated vascular strand.

Fig. 10. *A. balsamea* Mill.: two widely separated strands of vascular tissue and absence of thick-walled cells in pericyclic region.

Fig. 11. *A. homolepis* Sieb. & Zucc.: two separated vascular strands and abundant thick-walled cells in pericyclic region.

Fig. 12. *A. concolor* Lindl. & Gord., Jour. Hort. Soc. Lond. 5: 210 (1850). Colorado to southern and Lower California, northern continental Mexico and New Mexico.

Fig. 13. *A. cilicica* Carr., Conif. 229 (1855). Asia Minor (Cilicia), northern Syria.

Fig. 14. *A. Veitchii* Lindl., Gard. Chron. 23 (1861). Mountains of central Japan.

Fig. 15. *A. grandis* Lindl., Penny Cyclop. 1: 30 (1833). Vancouver Island to northern California, east to Montana, near the coast and in mountains.

Fig. 16. *A. Pinsapo* Boiss., Bibl. Univ. Genève 13: 167 (1838). Southern Spain.

Fig. 17. *A. Nordmanniana* Spach, Hist. Veg. Phan. 11: 418 (1842). Caucasus, Asia Minor, Greece.

Fig. 18. *A. Faxoniana* Rehd. & Wils., Sargent, Pl. Wils. 2: 42 (1914). High altitude forests in western China.

Fig. 19. *A. Pindrow* Spach, Hist. Veg. Phan. 11: 423 (1842). Western Himalaya, Kumaon to Kashmir.

Fig. 20. *A. nephrolepis* Maxim., Bull. Acad. Petersb. 10: 486 (1866). Eastern Siberia, northern China.

Fig. 21. *A. spectabilis* Spach., Hist. Veg. Phan. 11: 422 (1842). Sikkim and Bhutan Himalaya.

Fig. 22. *A. Forrestii* Craib, Notes Bot. Gard. Edin. 11: 279 (1920). Southwestern China at high altitudes.

Fig. 23. *A. Mariesii* Mast., Gard. Chron. 12: 789 (1879). Mountains of Japan.

Fig. 24. *A. sachalinensis* Mast., Gard. Chron. 12: 588 (1879). Northern Japan, Saghalin and Kurile Islands.

Fig. 25. *A. squamata* Mast., Gard. Chron. 39: 299 (1906). Western China at high altitudes.

Fig. 26. *A. homolepis* Sieb. & Zucc., Fl. Jap. 2: 17 (1842). Mountains of Japan.

Fig. 27. *A. venusta* K. Koch, Dendrol. 2 II: 210 (1873). Monterey County, California, at elevations of about 3000 feet.

Fig. 28. *A. cephalonica* Loud., Gard. Mag. 14: 81 (1838). Mountains of Greece.

Fig. 29. *A. recurvata* Mast., Jour. Linn. Soc. Bot. 39: 299 (1906). Western China.

Fig. 30. *A. sibirica* Ledeb., Fl. Alt. 4: 202 (1833). Northern Russia to Kamchatka, Altai Mountains, south to Turkestan and Manchuria.

Fig. 31. *A. arizonica* Merr., Proc. Biol. Soc. Washington 10: 116 (1896). Northern Arizona and northern New Mexico, southern Colorado.

Fig. 32. *A. Delavayi* Franch., Jour. de Bot. 13: 258 (1899). Western China at high altitudes.

Fig. 33. *A. nobilis* Lindl., Penny Cyclop. 1: 30 (1833). Washington to northern California.

Fig. 34. *A. holophylla* Maxim., Bull. Acad. Petersb. 10: 487. 1866. Manchuria and Korea.

Fig. 35. *A. firma* Sieb. & Zucc. Fl. Jap. ii 15t. 107 (1842). Japan up to 7000 feet elevation.

Fig. 36. *A. sutchuenensis* Rehd. & Wils. Sargent, Pl. Wils. 2: 48 (1914). Western China.

Fig. 37. *A. koreana* Wils., Jour. Arn. Arb. 1: 188 (1920). Mountains of Korea.

Fig. 38. *A. balsamea* Mill. Gard. Dict. ed. 8 n. 3 (1768). Labrador to West Virginia, west to Minnesota and Iowa.

Fig. 39. *A. lasiocarpa* Nutt. North American Sylva 3: 138 (1849). Alaska to Oregon, Utah and northern New Mexico.

Fig. 40. *A. alba* Mill. Gard. Dict. ed. 8. (1768). Mountains of central and southern Europe.

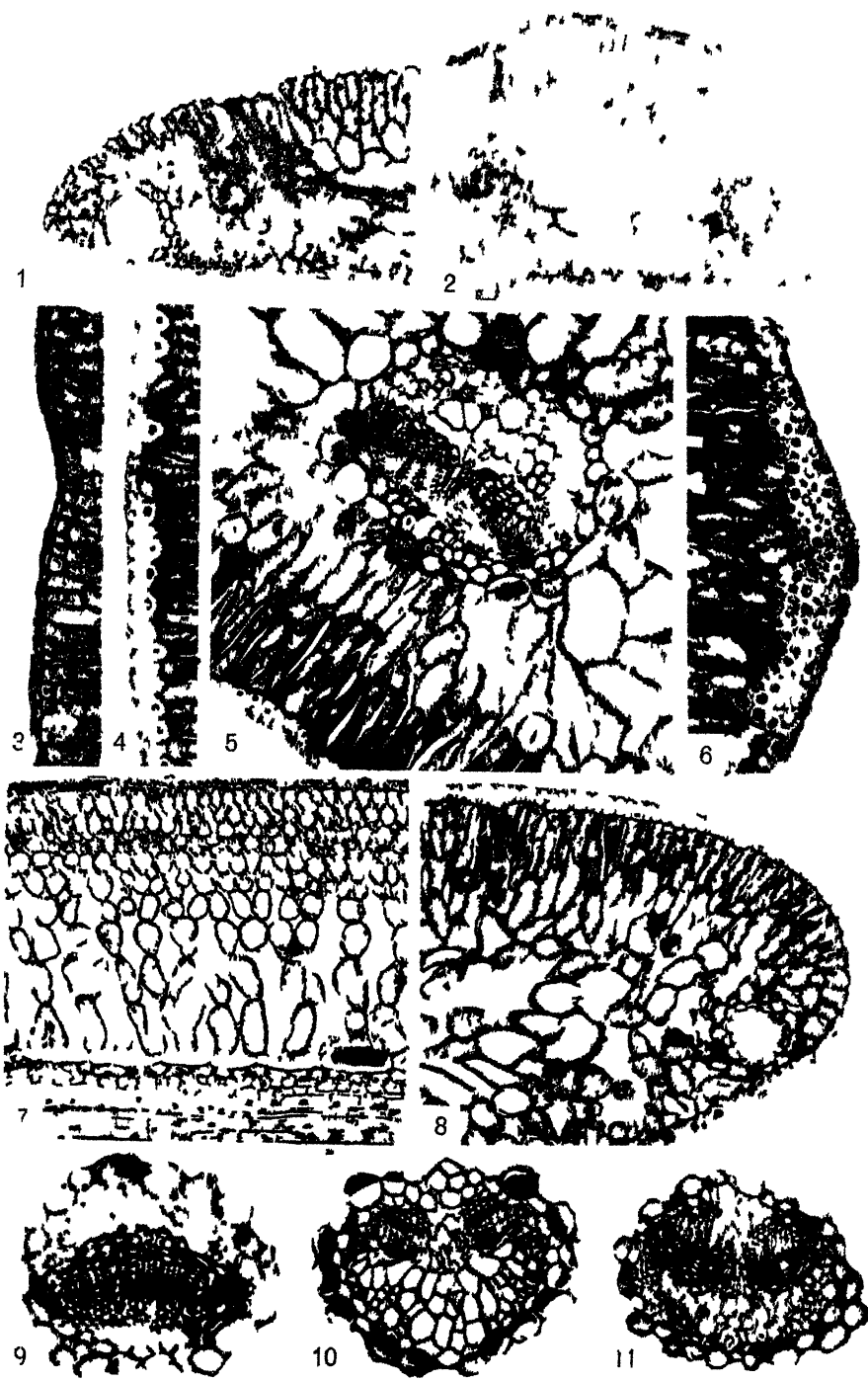
Fig. 41. *A. Fraseri* Poir., Lamarck Encyc. Method. Sup. v (1817). Alleghany Mountains, West Virginia to North Carolina and Tennessee.

Fig. 42. *A. magnifica* Murr., Proc. Roy. Hort. Soc. 3: 318 (1863). Oregon to California, 5000 to 10,000 feet elevation.

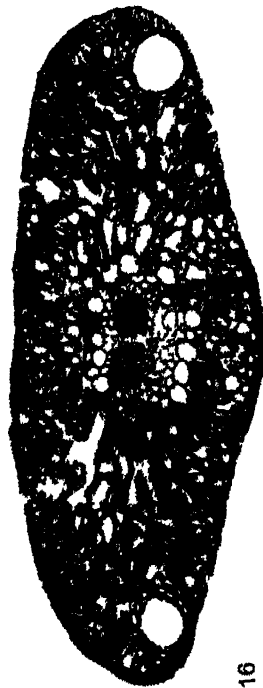
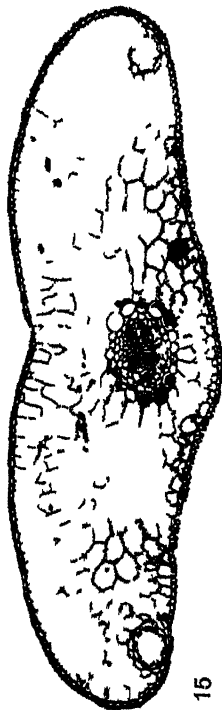
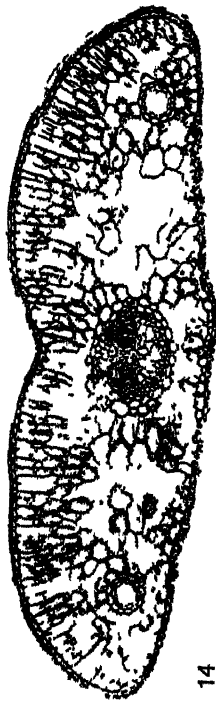
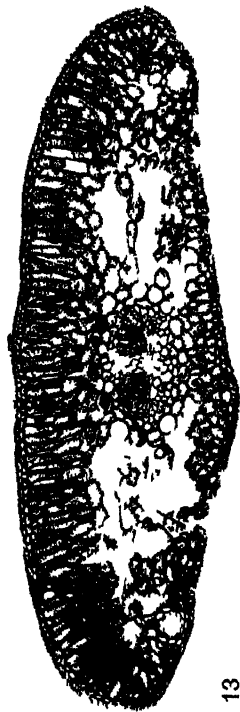
Fig. 43. *A. amabilis* Forb., Pin. Wob. 125 (1839). British Columbia and Alberta to Oregon.

Figures 1 to 11 represent magnifications of 75 to 100 diameters; all the others are about 50.

Photomicrographs of *A. Pindrow* and *A. spectabilis* are not typical of the species, being probably from fertile branches. On sterile branches, resin canals are normally marginal.



FALLING LEAF STRUCTURE OF ABIES





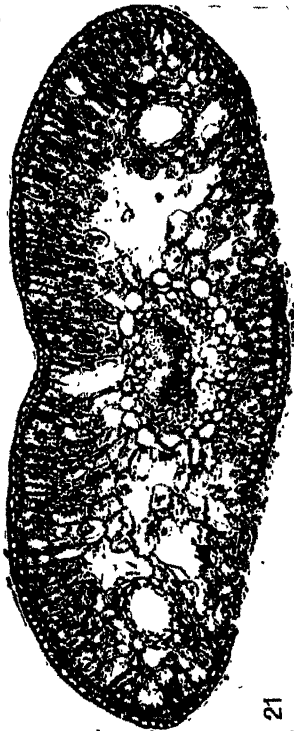
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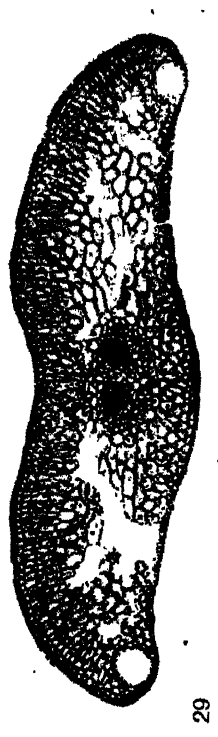
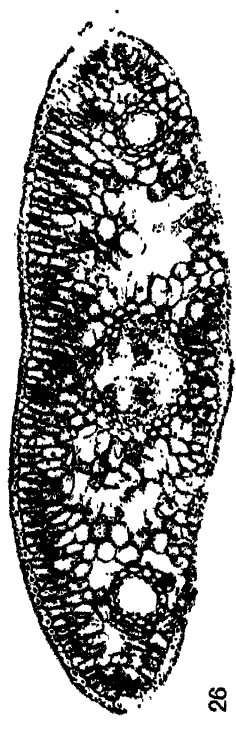
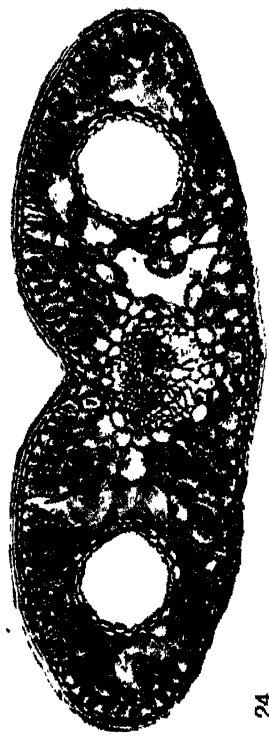
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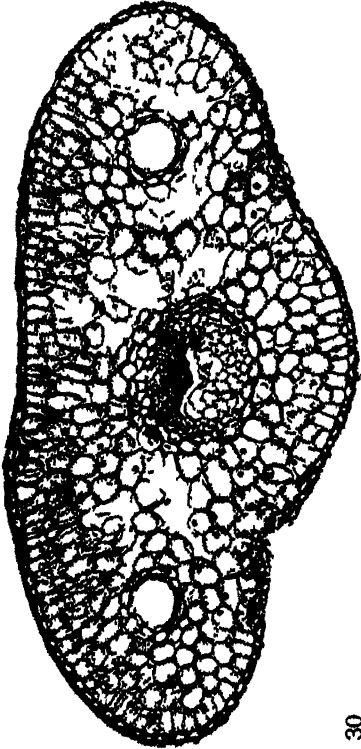
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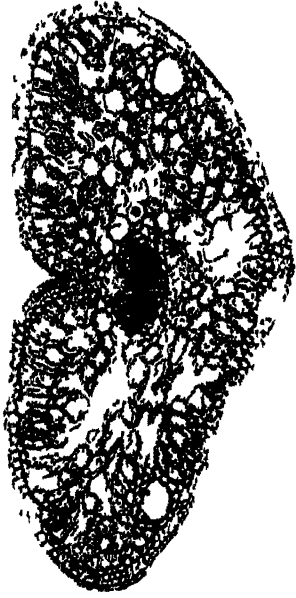
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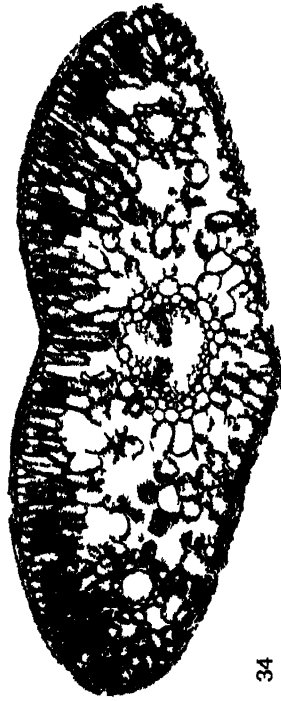
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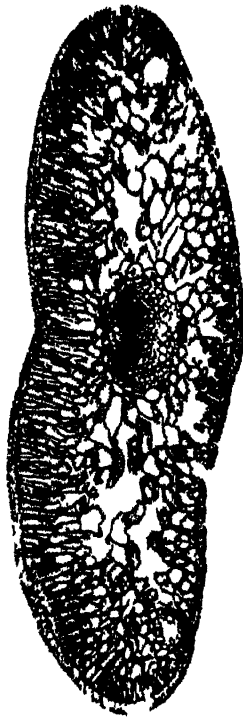
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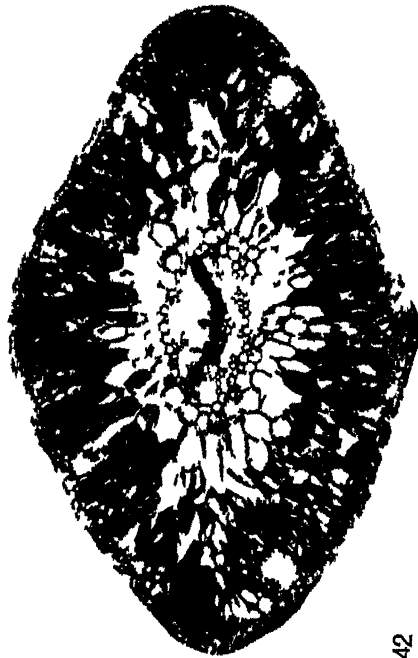
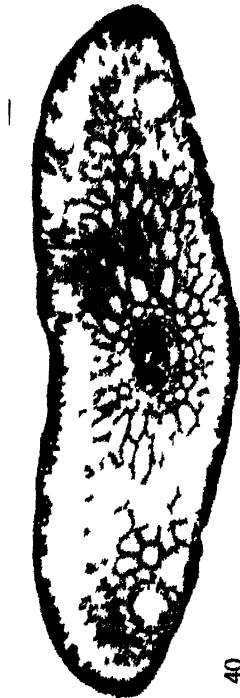
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FULLING LEAF STRUCTURE OF ABIES

INDEX TO AMERICAN BOTANICAL LITERATURE

1931-1934

The aim of this Index is to include all current botanical literature written by Americans, published in America, or based upon American material; the word America being used in the broadest sense.

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